

CHEMISTRY OF TAXANES AND TAXUS SPECIES

By

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This work is dedicated to my father whose untimely passing due to the disease that this work addresses on April 28, 1998 has left a deep void in my life that will never be filled. Although he would not be considered an educated man by most standards he taught me more than any textbook or professor ever could. I hoped he could be here when this work was completed but that was not to be. Nevertheless I hope that this accomplishment would make him as proud of me as I was of him. I love and miss you daddy.

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On more personal notes I first must give thanks to my wife, Amy, for all her support through this ordeal. Many times I knew she wanted me to stay home more often, many times I knew she was tired of living the impoverished life of a graduate student but through it all she remained faithful and supportive and for this I owe her a large debt of gratitude. I would also like to acknowledge the family of my wife for all their financial support that was given to us when we needed it. This also holds true for the families of my sisters Melinda and Teresa. I am very grateful to you all for your support. Last of all I would like to acknowledge my parents. It has been said that there is only one point in a persons life in which he is totally at the mercy of fortune, this is when he is born. I must say that I was blessed with parents who knew love and showed it to each other as well as myself, and that they showed this love by instilling in me discipline, integrity, and respect. I certainly could not be here today if it were not for them.

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ABBREVIATIONS

Ac - acetate
Bn - benzyl
Bz - benzoate
CIMS - chemical ionization mass spectroscopy
DCC - dicyclohexylcarbodiimide
DDQ - 2, 3-dichloro-5, 6-dicyano-1, 4-benzoquinone
DMF - dimethylformamide
DMSO - dimethyl sulfoxide
EIMS - electron impact mass spectroscopy
FABMS - fast atom bombardment mass spectroscopy
HMBC - heteronuclear multiple bond correlation
HPLC - high pressure liquid chromatography
LAH - lithium aluminum anhydride
LDA - lithium diisopropylamide
NMR - nuclear magnetic resonance
NOE - nuclear Overhauser effect
PDC - pyridinium dichromate
PTSA - para-toluene sulphonic acid
RaNi - rainy nickel
TBS - tert-butyl dimethyl silyl
TES - triethyl silyl
Tf - triflate
TLC - thin layer chromatography
TMEDA - tetramethylethylenediamine

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Chairman: Koppaka V. Rao (deceased)
Co-Chairman: John Perrin
Major Department: Medicinal Chemistry

The chemistry of both taxane diterpenoids and *Taxus* species are studied. Several taxanes are isolation from both *Taxus brevifolia* and *Taxus floridana*. In addition to these taxanes several non-taxane compounds are also isolated. One of these, trans-2, 6-dimethoxycinnamaldehyde is a novel structure that has been synthesized. Another non-taxane, taxamairin B, which belongs to rare class of diterpenes, is synthesized by a more efficient route than was reported in the literature. Nitrate ester forming reactions are also studied with paclitaxel and closely related analogues. It is shown that this reaction is regioselective in many cases and thus illustrates the potential use of nitrate esters as protecting groups in taxane chemistry. Also several unexpected reactions of these nitrate esters are explored including an unusually rearrangement in which the nitrated paclitaxel

side chain reacts in mild base to yield the corresponding baccatin III and dibenzamide. Finally, a series of potentially more water soluble paclitaxel analogues are prepared by oxidizing the naturally occurring 10-deacetyl paclitaxel-7-xyloside with periodate and then reacting the resulting dialdehyde with amines and carbon nucleophiles. These compounds are also tested for cytotoxicity in the L1210 assay system. Although these compounds are not as active as paclitaxel most are more active than the xyloside from which they are obtained.

CHAPTER 1 HISTORY AND BACKGROUND OF PACLITAXEL

Introduction

Paclitaxel (TaxolTM) (1) is a potent antitumor agent that was originally isolated from the Pacific yew tree, *Taxus brevifolia*, by Wall and Wani in 1971 (Wani et al., 1971). The structure of paclitaxel is that of a tetracyclic diterpene ester (Figure 1-1). Its structure has several unusual features including an oxetane ring, an 8-membered B-ring, a bridgehead double bond, 11 asymmetric centers, and a N-substituted phenylisoserine ester. This structure has provided a great challenge to synthetic organic chemists since its elucidation but was conquered initially by two groups simultaneously and by other groups since (Nicolaou et al., 1994; Holton et al., 1994a; Holton et al., 1994b). The ongoing phytochemical study of *Taxus brevifolia* as well as other *Taxus sp.* has yielded many taxanes other than paclitaxel. Compounds with rearranged ring systems, various types of esters, as well as glycosides have been isolated and several reviews have been published (Kingston et al., 1993; Das et al., 1995; Appendino, 1995).

Early Work and Structural Elucidation

The pioneering work concerning paclitaxel began in the late 1950s when the National Cancer Institute started a screening program of plant extracts using tumor

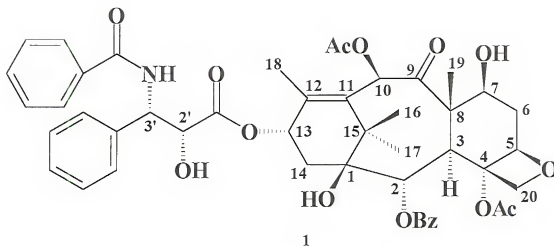


Figure 1-1: Structure of Paclitaxel

systems models *in vivo* and tumor cell lines. From these studies the stem bark extract of the Pacific yew tree was shown to display cytotoxicity in the KB assay and also activity against carcinosarcoma in rats and leukemia in mice. In connection with this NCI screening program, Wall and his collaborators studied the *in vitro* bioassay guided fractionation of the active extract and in 1969 paclitaxel was isolated and shown to be the most active constituent of the extract. This isolation was carried out by extracting the dried stem bark with 95% ethanol. The extract was then partitioned between water and 4 : 1 chloroform : methanol. The organic layer was evaporated to a solid and purified by a 3-step Craig countercurrent distribution method which yielded paclitaxel in a yield of 0.004%. As soon as paclitaxel had been isolated in pure form, the structure of the compound was investigated using available spectroscopic methods. Although methods for ultraviolet, infrared, and mass spectrometry were at a reasonably advanced stage in the

late 1960s, NMR was relatively primitive compared to the sophisticated instrumentation and procedures now available. Some of the physical and chemical properties of paclitaxel are shown in Table 1-1 and the ^1H NMR spectrum is shown in Figure 1-2.

Table 1-1: Physical and Chemical Properties of Paclitaxel

1.) Needles from 50% aqueous methanol or ether
2.) mp 213-216° C
3.) $[\alpha]_D^{20}$ -49.6° (MeOH)
4.) Unstable towards mineral acid and base
5.) Forms mono and diacetate
6.) Analysis Calcd. for $\text{C}_{47}\text{H}_{51}\text{NO}_{14}$: C, 66.11; H, 6.20; N, 1.64 Found: C, 65.98; H, 6.10; N, 1.57. Required m/z 853. Found m/z 853
7.) UV λ_{max} (MeOH) 227 nm (ϵ 29,800)

It was evident by this time that paclitaxel probably contained the taxane skeleton. A number of taxane derivatives had been reported in previous literature. It was evident that paclitaxel was more complex than previously reported taxanes since its molecular weight from high resolution mass spectrometry was $\text{C}_{47}\text{H}_{51}\text{NO}_{14}$, corresponding to a molecular weight of 853. The evidence then indicated that paclitaxel was comprised of a taxane nucleus to which an ester was attached, as preliminary experiments indicated that an ester was easily cleaved from the rest of the molecule. Attempts were made to prepare crystalline halogenated derivatives of paclitaxel, however none had properties suitable for x-ray analysis. Paclitaxel was therefore subjected to a mild base catalyzed methanolysis at 0° C, which yielded a nitrogen containing α -hydroxy methyl ester, $\text{C}_{17}\text{H}_{17}\text{NO}_4$, a tetraol, $\text{C}_{29}\text{H}_{36}\text{O}_{10}$, and methyl acetate. The methyl ester thus obtained by the mild methanolysis procedure was converted to a parabromobenzoate ester (**2**) and characterized by x-ray analysis as $\text{C}_{24}\text{H}_{20}\text{BrNO}_5$ with the structure shown in Figure 1-2. The ester may be

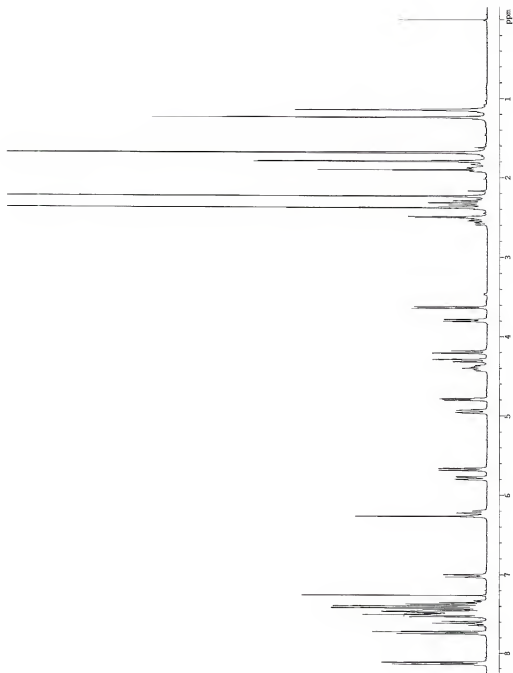
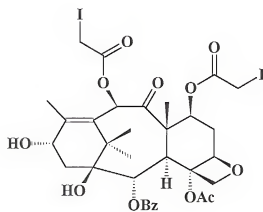
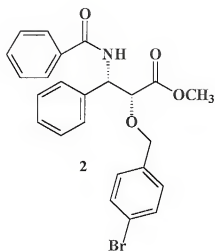


Figure 1-2: ^1H NMR Spectrum of Paclitaxel



3

**Figure 1-3: Halogenated Products of Methanolysis
Used for X-Ray Crystallography**

regarded as an N-benzoyl derivative of (2R, 3S)-3-phenylisoserine. The tetraol formed by the methanolysis of paclitaxel was converted to a bisiodoacetate (**3**), C₃₃H₃₈I₂O₁₂, which again received x-ray analysis. The structure is shown in Figure 1-3.

Since the ester could have originally been joined to hydroxyl groups at either C-7, C-10, or C-13, it was necessary to establish at which of these hydroxyl moieties the ester had originally been located. When paclitaxel was oxidized with MnO_2 under neutral conditions, no reaction occurred. However, MnO_2 oxidation of paclitaxel under alkaline conditions smoothly yielded a reaction product (4) with the structure shown in Figure 1-4. It is evident that MnO_2 oxidation of paclitaxel under neutral conditions did not effect the hydroxyl groups available for oxidation at C-7 and C-2'. When paclitaxel was oxidized with alkaline MnO_2 an analogue of baccatin III with a conjugated carbonyl moiety as shown in Figure 1-4 was obtained. It is well known that MnO_2 oxidation of allylic hydroxyl groups under alkaline conditions smoothly forms the corresponding conjugated ketone. This reaction in conjunction with the x-ray structure determination of the structures of the ester and taxane moieties established the structure of paclitaxel (Wani et al., 1971).

Mechanism of Action

Although paclitaxel displayed good activity against human tumor xenographs and murine B16 melanoma, its cytotoxic properties were not very different from other drugs being tested during the 1970s. Rather, its attraction to pharmacologists was its unique structure which suggested the possibility of a novel mechanism for an anti-tumor drug. This mechanism was subsequently identified in 1979 by Horwitz and collaborators (Schiff et al., 1979). Paclitaxel proved to be a potent inhibitor of eukaryotic cell replication, blocking cells in the late G2-M phase of the cell cycle. It is an unusual mitotic inhibitor

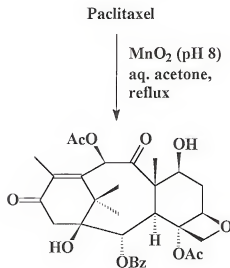
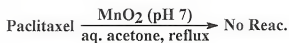


Figure 1-4: Neutral and Alkaline Oxidation of Paclitaxel

because, unlike the vinca alkaloids and colchicine which inhibit microtubule assembly, it promotes the formation of discrete bundles of stable microtubules that result from the reorganization of the microtubule cytoskeleton. The novel characteristic of paclitaxel is its ability to polymerize tubulin *in vitro* in the absence of guanosine 5'-triphosphate (GTP), which is normally required for tubulin assembly.

Total Synthesis

As mentioned, the total synthesis of paclitaxel has recently been achieved by various groups, however one group alone cannot claim to be the first to accomplish this

daunting task as Nicolaou and Holton published their work simultaneously in two separate journals. Their respective works are now briefly described.

Relying on the previous work of other groups as well as his own studies, Nicolaou envisioned the late formation of the oxetane (D) ring, oxygenation/reduction of the C-13 position, and attachment of the side chain. Thus, the problem was perceived as limited to the assembly of paclitaxel's ABC ring system in either its fully functionalized form or a form that would serve as its progenitor. Figure 1-5 displays the retrosynthetic analysis involving the bond disconnections on which the synthetic strategy was based. Thus, in the synthetic direction the following key operations were performed: 1) two fragments (7 and 8) representing precursors to rings A and C were coupled by a Shapiro reaction and a McMurry coupling to assemble the ABC ring skeleton; 2) installment of the oxetane ring; 3) addition of the various substituents around the peripheries of rings B and C; 4) oxygenation at C-13; and 5) esterification to attach the side chain. Both precursors to rings A and C were made possible using the Diels-Alder transform which led to starting materials that were either commercially available or known in the literature.

In contrast to the convergent synthesis by Nicolaou, Holton took a more linear approach. The facile epimerization of paclitaxel at C-7 is well documented, and has been postulated to occur via a retroaldol-aldol process. Holton chose therefore to pursue a synthetic strategy in which this stereocenter would be introduced at an early stage and carried throughout most of the synthesis in the absence of a C-9 carbonyl group, thereby avoiding epimerization. Thus, his route to paclitaxel proceeds retrosynthetically through the C-7 protected baccatin III **13** to the tricyclic ketone **14**, which arises from C ring

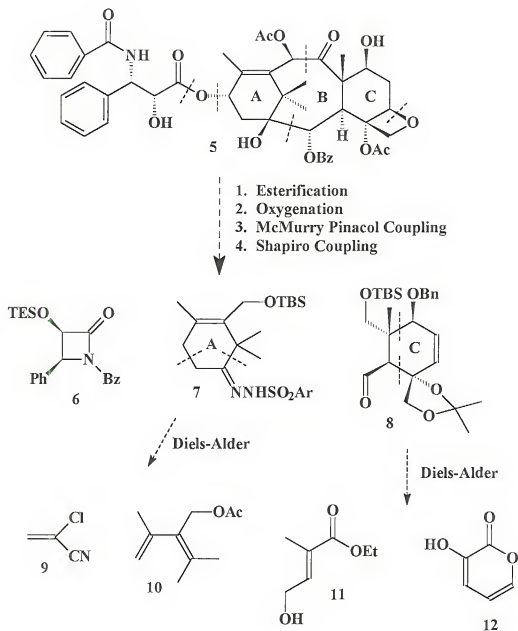


Figure 1-5: Nicolaou Synthesis of Paclitaxel

closure of a precursor properly functionalized at C-1, C-2, C-3, C-7, and C-8 (15).

Synthesis of this precursor was made possible by conformational control of the eight

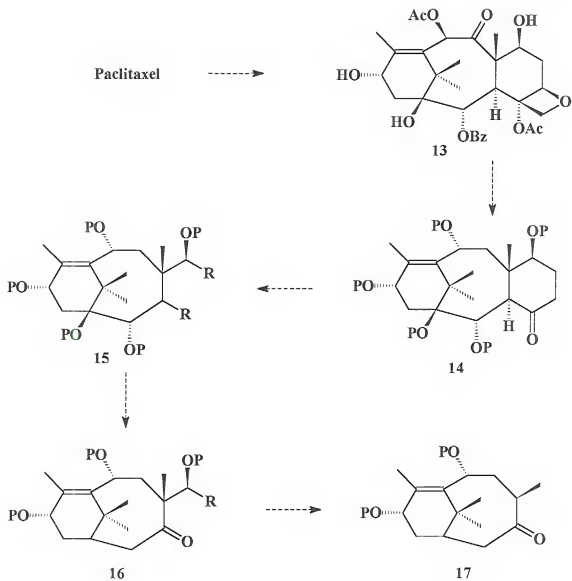


Figure 1-6: Holton Synthesis of Paclitaxel

membered B ring, via ketone 16. Ketone 16 was projected to arise from an aldol condensation of the enolate of ketone 17, the formation of which would be made possible

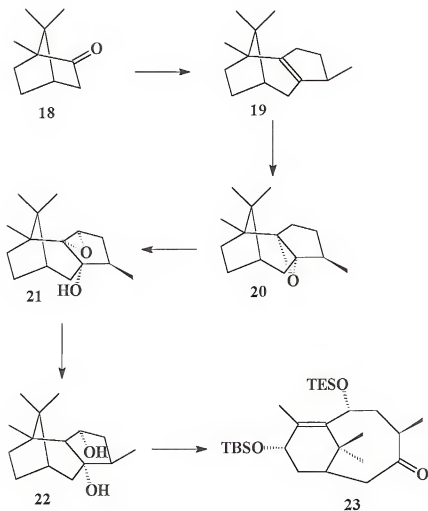


Figure 1-7: Holton Synthesis of Paclitaxel

by conformational control exerted by the C-10 α group. This scheme is shown in Figure 1-6. The synthesis of this starting ketone begins with camphor (18). Camphor is converted to β -patchouline (19) and its epoxide also known as "Patchino" (20). Patchino is then converted to epoxide 21 and then rearranged to diol 22 which is followed by epoxy alcohol fragmentation to give the starting ketone 23 as is shown in Figure 1-7.

Although there have been many publications concerning the synthesis of the phenyl isoserine side chain, the most common and that which was used in both of these methods is using the β -lactam 6 in Figure 1-5.

Structure-Activity Relationships

During the past decade a tremendous amount of work has been performed to determine what parts of the structure of paclitaxel are necessary to illicit biological activity and reviews have been published (Commercon et al., 1995; Chen & Farina, 1995; Kingston, 1995; Ojima et al., 1995; Georg et al., 1995). The structure of paclitaxel can be divided into 3 sections when discussing structure-activity relationships and these are: 1) the N-benzoyl phenylisoserine side chain; 2) the southern hemisphere including C-2, C-4, and the oxetane ring; and 3) the northern hemisphere including C-7, C-9, and C-10.

The side chain plays a major role in the biological functions of this antitumor agent and without the side chain the resulting baccatin III is inactive. Protection of the C-2' hydroxyl group results in major loss of activity in tubulin assays but if the group is labile (acetate) then the activity remains in cell culture presumably acting as a prodrug. Structural modifications of the paclitaxel side chain have been reported by several groups. These studies reveal a number of interesting features and important findings include the following: 1) the C-3' amide group is critical although the amide's aryl group may be substituted by other aryl or alkyl groups; 2) the C-3' aryl group is required since replacement by a methyl group reduces activity but, if larger alkyl groups are used, the activity remains; 3) the C-3' bound nitrogen can be replaced by an oxygen atom without

significant loss of activity; 4) one of the C-2' or C-3' polar functions can be removed without significant effect, but the removal of both or interchange of their positions causes dramatic loss of activity and; 5) the (2'S, 3'R) naturally occurring isomer is the most active of the four possible isomers.

Concerning the southern hemisphere of the structure, this area is also very important in terms of biological activity. First of all the oxetane ring is necessary for activity. Structural and molecular modeling studies show that this 4-membered ring is involved in a conformational lock of the diterpene skeleton and the C-13 side chain through a pseudo chair conformation of ring C. The C-2 benzoyl group is also necessary for activity as the C-2 debenzoyl paclitaxel showed little *in vitro* cytotoxicity; however, some groups have shown that modified benzoyl groups or aryl acyl groups do retain the cytotoxicity. The C-4 acetate is not as important as the C-2 benzoate in that if the acetate is removed the activity is reduced only slightly.

The northern hemisphere is the least sensitive part of the paclitaxel structure. The C-7 hydroxyl may be esterified, epimerized, or removed without significant loss of activity. Specifically a xylosyl group at C-7 actually increases the activity in the tubulin binding assay but leads to decreased activity in cell culture. Presumably there is a transport problem associated with the xyloside that causes this decreased activity. The C-9 keto group may be reduced which actually slightly improves activity and the C-10 acetyl or acetoxy group may be removed without significant loss of activity.

It should also be said that contraction of the A ring does not reduce the tubulin-disassembly inhibition activity very much in spite of the significant structural change

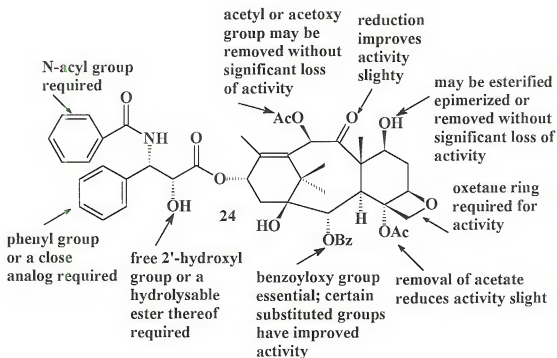


Figure 1-8: Structure-Activity Relationships

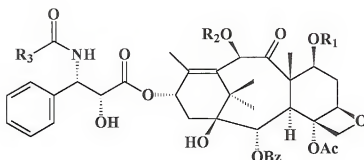
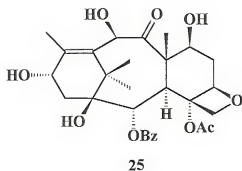
implied by this conversion. These analogues, however, are much less cytotoxic in cell culture which may be due to the instability of the A ring contracted analogues in cell culture media or to its failure to enter the cell. A summary of structure-activity relationships is shown in Figure 1-8.

CHAPTER 2 ISOLATION OF TAXOID AND NON-TAXOID COMPOUNDS FROM TAXUS SPECIES

Large Scale Isolation Process

Although paclitaxel is one of the most promising anti-tumor drugs to receive FDA approval in many years, it has been beset with many problems not the least of which is adequate production of the drug. The original, and until recently major source of the compound was the bark of the Pacific yew (*Taxus brevifolia*), from which paclitaxel was isolated in a yield of 0.01-0.013% on a large scale. Although several related taxanes that can serve as precursors for the semi-synthesis of paclitaxel, for example, 10-deacetyl baccatin III (25), co-occur in the bark with paclitaxel, there are no reports to indicate that these are being isolated from the bark on a large scale. Thus the low yields of paclitaxel realized by the original process, the apparent unavailability of other useful taxanes analogues, and the environmental concerns raised by the need to cut the slow-growing yew trees for harvesting the bark, are some of the reasons why the bark is no longer considered an attractive source for the large scale production of paclitaxel.

Among the alternatives that are being actively studied are the following: 1) isolation of 10-deacetyl baccatin III from the European yew (*Taxus baccata*) and its semi-



- 26 $R_1 = H, R_2 = Ac, R_3 = \text{phenyl}$
 27 $R_1 = \beta\text{-xylosyl}, R_2 = H, R_3 = \text{tiglyl}$
 28 $R_1 = \beta\text{-xylosyl}, R_2 = H, R_3 = \text{phenyl}$
 29 $R_1 = \beta\text{-xylosyl}, R_2 = H, R_3 = n\text{-pentyl}$
 30 $R_1 = \beta\text{-xylosyl}, R_2 = Ac, R_3 = \text{phenyl}$
 31 $R_1 = H, R_2 = H, R_3 = \text{phenyl}$
 32 $R_1 = H, R_2 = Ac, R_3 = \text{tiglyl}$

Figure 2-1: Structure of Major Taxanes

synthetic conversion to paclitaxel and 2) large-scale cultivation of the ornamental yew (*Taxus media* Hicksii) and isolation of paclitaxel from its needles/twigs. Among the future alternatives are total synthesis, of which various schemes have been published, and isolation from large-scale plant cell culture.

In recent years this laboratory has developed a large-scale isolation procedure using a single reverse-phase column (Rao, 1993; Rao et al. 1995). This procedure has several advantages over other published procedures some of which are that it is much simpler, gives higher yields of paclitaxel, and yields several other taxanes which can be converted to paclitaxel. Specifically, the following yields are obtained for the major compounds from the bark: paclitaxel (**26**) (0.04%), 10-deacetyl baccatin III (**25**) (0.02%), 10-deacetyl paclitaxel-7- β -xyloside (**28**) (0.1%), 10-deacetyl paclitaxel-C-7- β -xyloside (**29**) (0.04%), 10-deacetyl cephalomannine-7- β -xyloside (**27**) (0.006%), paclitaxel-7- β -xyloside (**30**) (0.008%), 10-deacetyl paclitaxel (**31**) (0.008%), and cephalomannine (**32**) (0.004%) (Figure 2-1). The procedure for this process is defined below and in Figure 2-2.

Air dried yew bark (200-250 lbs.) was extracted with 100 gallons of methanol in a batch process a total of three times with each extraction lasting one day. The pooled methanol extracts were concentrated under reduced pressure ($<30^{\circ}$ C) using a semi-continuously operated still until the volume of the concentrate reached 20-25 gallons. Extraction of the concentrated methanolic extract with chloroform was performed in 50-100 gallon tanks equipped with an air-driven stirrer. The concentrate was stirred with water and chloroform for about 30 minutes, then 2-14 hours were necessary to allow for any emulsion to clear. The chloroform layer was drained off from the bottom and the water layer was extracted two additional times. The pooled chloroform layers were concentrated under a vacuum to a thick syrup which was poured into glass trays and converted to powder using a vacuum oven at $35-40^{\circ}$ C. The powder was obtained in a yield of 18-26 g per kg of the bark.

For chromatography, stainless steel columns either 4" x 4' or 6" x 6' were used. The columns were packed with C-18 bonded silica as a slurry in methanol. Approximately 3-4 kg and 12-13 kg of silica were used with the 4" and 6" columns, respectively. After a thorough wash with methanol, the columns were equilibrated with 25% acetonitrile in water. For running the 6" diameter column, the powder from the chloroform extract (2-2.5 kg) was dissolved in acetonitrile (5 l) and while this mixture was being stirred with equilibrated C-18 silica (1-2 l), it was diluted with water to make 20 l. The mixture was then allowed to stand for 15-30 minutes and the clear supernatant siphoned off into another container. The slurry was applied to the column, followed by part of the supernatant, after which the column was sealed. The remaining supernatant was pumped into the column using a diaphragm metering pump maintaining a pressure of 30-80 psi. After the sample had been pumped onto the column it was eluted with a step gradient of 35, 40, 45, and 50% acetonitrile in water. The change in solvent was dictated by the results of the TLC and HPLC of the fractions but usually 40-50 l of each solvent was used. After this, the column was washed with methanol, followed by a mixture of ethyl acetate and ligroin until the effluent was nearly colorless. Following this, the column was again washed with methanol and equilibrated with 25% acetonitrile in water. The column fractions (about 2 l each) were allowed to stand at room temperature for 2-8 days, by which time crystals appeared in many. Soon after, the crystals were filtered, analyzed for purity and composition by HPLC, and recrystallized from the appropriate solvent.

In terms of the elution sequence of the taxanes, the earliest taxane to appear was 10-deacetyl baccatin III (25) which crystallized from the fractions eluted by 35%

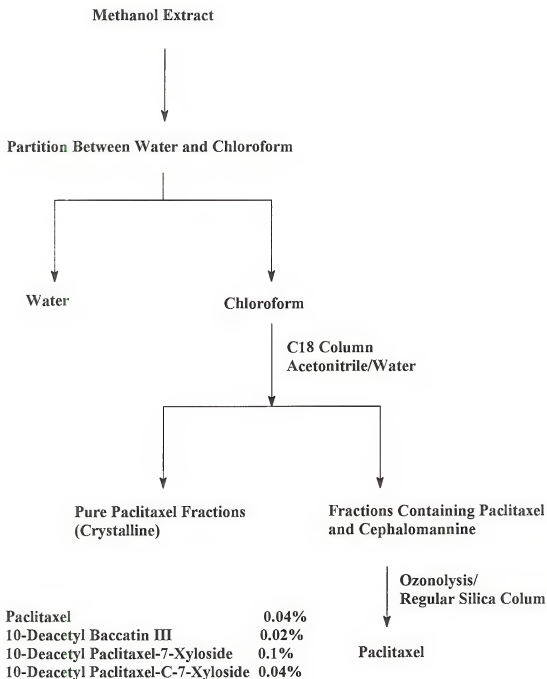


Figure 2-2: Reverse-Phase Isolation of Taxanes

acetonitrile in water. The next group of taxanes to be eluted were the various xylosidic taxanes; 10-deacetyl cephalomannine-7- β -xyloside (27), 10-deacetyl paclitaxel-7- β -xyloside (28), 10-deacetyl paclitaxel-C-7- β -xyloside (29), and paclitaxel-7- β -xyloside (30). Of these the first two were well separated. As the elution of 10-deacetyl paclitaxel-7- β -xyloside was nearing completion, 10-deacetyl paclitaxel-C-7- β -xyloside started to elute. Halfway through its elution, paclitaxel-7- β -xyloside and 10-deacetyl paclitaxel (31) started to co-elute. These last two compounds also crystallized together, however separation was readily achieved by running the mixture through a regular silica column using 0-5% methanol in chloroform as solvent.

Continued elution of the column with 50% acetonitrile in water gave cephalomannine (32), followed closely by paclitaxel (26). The earlier part of the band contained mixtures of the two, but the later fractions contained mostly paclitaxel which could be recrystallized. The fractions that contained the mixture were combined and dried to a solid. This solid was then subjected to ozonolysis at -78°C for 45 minutes. This process converted the cephalomannine to the keto-amide 34 but did not disturb paclitaxel (Figure 2-3). After workup this material was run through a regular silica column with 0-5% acetone in chloroform and the paclitaxel was isolated. It should be pointed out that this process was necessary because paclitaxel and cephalomannine cannot be separated on regular-phase silica.

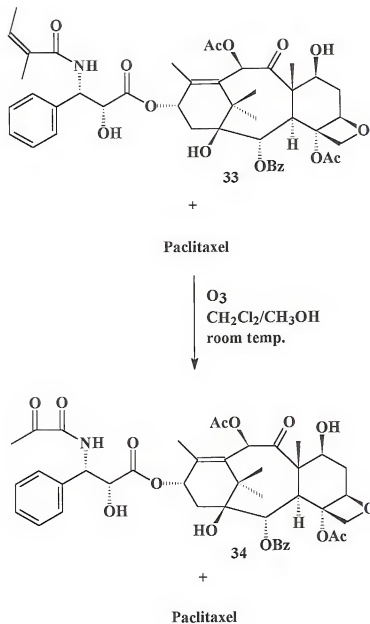


Figure 2-3: Ozonolysis of Cephalomannine/Paclitaxel Mixture

Isolation of Minor Compounds from the Bark of *Taxus brevifolia*

Obviously the above process was only used to isolate major compounds; however, many minor compounds exist in the filtrates or in the in-between fractions. A TLC analysis of the filtrates from the region between 10-deacetyl baccatin III and 10-deacetyl paclitaxel (xyloside fractions) showed many interesting compounds, the identity of which could not be determined by comparison with available standards. This material was therefore concentrated to a solid and rechromatographed on regular-phase silica using an elution system of 0-5% acetone in dichloromethane to 0-5% methanol in 5% acetone/dichloromethane.

The first compound to be eluted was 1 β -hydroxy baccatin I (35). This compound has been known for quite some time and was first isolated from *Taxus baccata* in 1970 (Della Casa De Marcano & Halsall, 1970). This compound belongs to the baccatin I sub-family because it contains a C-4-C-20 epoxide as opposed to an oxetane ring. Baccatin VI (36) was eluted next and is another well known taxane isolated for the first time from *Taxus baccata* in 1975 (Della Casa De Marcano & Halsall, 1975). This compound is so-named because it is esterified at C-9 as opposed to paclitaxel/10-deacetyl baccatin III which have a C-9 ketone (Figure 2-4).

The next compound to be eluted was 1 β -hydroxy-7-deacetyl baccatin I (37). This compound was recently isolated from the needles of *Taxus brevifolia* (Chu et al., 1993) and has been reported to undergo an acetyl migration from C-9 to C-7 when kept in solution. Indeed, this compound did form another spot on TLC when left in solution;

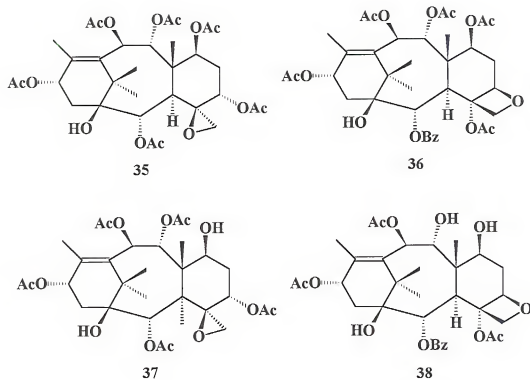


Figure 2-4: Compounds from the Bark of *Taxus brevifolia*

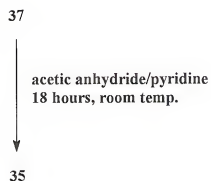
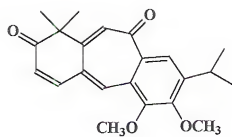


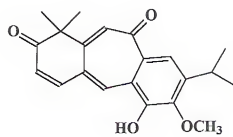
Figure 2-5: Acetylation of 1 β -Hydroxy-7-Deacetyl Baccatin I

however, no attempt was made to determine if this new compound was the C-7-acetyl, C-9-hydroxy isomer. To further confirm its structure **37** was acetylated with acetic anhydride and pyridine and the product matched 1 β -hydroxy baccatin I (**35**) in every way (Figure 2-5). Finally 9-dihydro-13-acetyl baccatin III (**38**) was eluted as determined by NMR spectroscopy (Figure 2-4). This compound was isolated earlier from the needles of *Taxus canadensis* (Gunawardana et al., 1992), but the current isolation is the first from the bark of *Taxus brevifolia*. This compound has received much attention from Abbott Laboratories as a possible precursor to their own paclitaxel analogue.

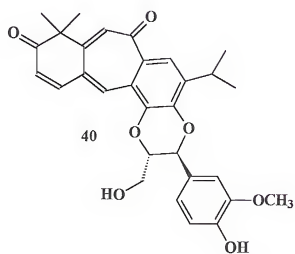
In addition to this work on the pre-paclitaxel fractions another interesting compound was isolated while attempting to obtain more paclitaxel from the filtrates of paclitaxel-containing reverse-phase fractions. This crystalline compound was eluted with a solvent mixture of 2% acetone in dichloromethane and was given the name brevixanthane because of its yellow color. Based on ^1H and ^{13}C NMR spectra it was quickly concluded that brevixanthane belonged to a rare group of diterpenes known as 9(10 \rightarrow 20)-abeo-abietane diterpenoids that have previously been isolated from *Taxus* species. These diterpenes consist of a 6, 7, 6 tricyclic carbon ring system with ring C being aromatic and are a novel diterpene structural class. The only other members of this group include taxamairin A (**38**) and B (**39**) from *Taxus chinensis* var. *Mairei* (Liang et al., 1987) and brevixin (**40**) from *Taxus brevifolia* which contains a C₆-C₃ side chain (Arslanian et al., 1995) (Figure 2-6). Initially, we thought this compound may be novel based on its ^1H NMR spectrum. Brevixanthane contained only one methoxyl which had a chemical shift of 3.89 ppm while the methoxyl of taxamairin A was reported to be at 3.99 ppm. All other



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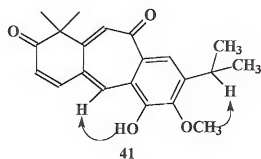


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Figure 2-6: Structure of Abeo-Abietane Diterpenoids



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Figure 2-7: NOE Correlations of Taxamairin A

chemical shifts were almost identical. Thus, it was speculated that the methoxyl/hydroxyl positions may be the reverse of that of taxamairin A. However, it was then observed that the UV spectrum of brevixanthane was identical to that reported for taxamairin A while that of taxamairin B was reported to be completely different. This was confirmed by synthesizing taxamairin B from brevixanthane with dimethylsulfate. Thus, since the UV spectra of taxamairins A and B are so different it stands to reason that the UV spectra of taxamairin A and brevixanthane should also be different if they were different structures. This was not the case. To solve this question of structure ^1H NMR NOE experiments were performed. These experiments illustrated that if the methoxyl methyl of brevixanthane is irradiated then the phenolic proton and the isopropyl methyne proton are enhanced proving the proximity of the methoxyl to the isopropyl group. Also when the phenol proton was irradiated the methoxyl protons and the C-4 proton was enhanced proving a close proximity between the phenolic group with and carbon 4 of the B ring (Figure 2-7). Thus, it is concluded that brevixanthane was the same compound as taxamairin A.

Synthesis of Taxamairin B

The total synthesis of taxamairin diterpenes has been accomplished by one other group (Wang & Pan, 1995a; Wang et al., 1995b). The synthetic strategy which was used by this group was derived from the retrosynthetic analysis as outlined in Figure 2-8. Ketone **45** was the key synthetic intermediate because it contains the entire carboncyclic framework of taxamairin B. Thus the A ring precursor (**49**) was readily obtained from 1,3

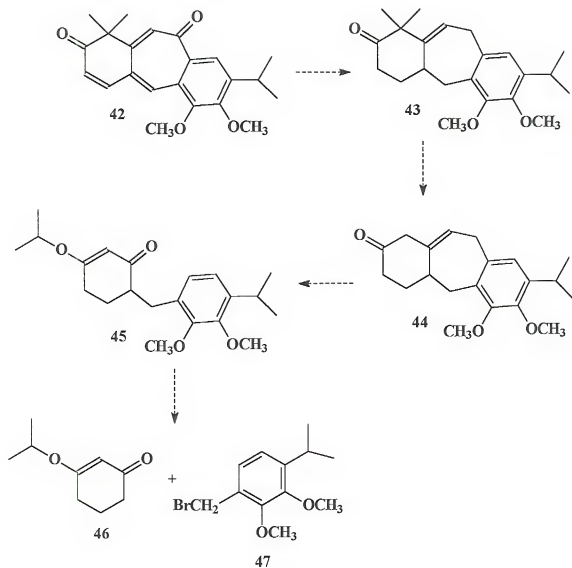


Figure 2-8: Retrosynthetic Analysis of Taxamairin B

-cyclohexanedione (48) by azeotropic removal of water from a benzene/hexane/isopropyl alcohol solution with PTSA as catalyst in a yield of 95% (Figure 2-9). The synthesis of the C ring was begun by oxidizing *o*-vanillin (50) with AgO to give the carboxylic acid. This phenolic acid was then dimethylated using dimethylsulfate to yield the methyl ester (51). This is then treated with two equivalents of methyl magnesium bromide to yield the

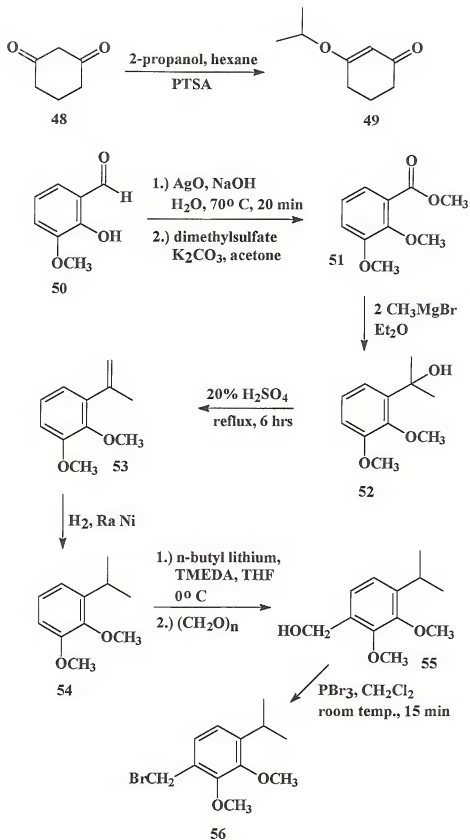


Figure 2-9: Literature Synthesis of Taxamairin B

tertiary alcohol (**52**). Dehydration was then achieved by heating with 20% H_2SO_4 . The resulting olefin (**53**) was then reduced with RaNi and H_2 . A hydroxymethyl group was attached ortho to the methoxyl by treating **54** with *n*-butyl lithium in THF and TMEDA and later addition of paraformaldehyde. The hydroxymethyl function was converted to a bromomethyl function using PBr_3 in dichloromethane. This bromo compound (**56**) then underwent nucleophilic substitution with ketone **49** and LDA as the base to yield ketone **57** (Figure 2-10). Ketone **57** was treated with vinyl magnesium bromide and the B ring was closed by a Friedel-Crafts alkylation using $\text{BF}_3\cdot\text{OEt}_2$. Finally the gem-dimethyls were attached using potassium *t*-butoxide and methyl iodide to produce the key intermediate **60** in which the olefin bond moved into the cycloheptane ring. Oxidation of the allylic and benzylic methylene group to produce the ketone at C-1 was achieved by using excess aqueous 75% *t*-butyl hydroperoxide and catalytic amounts of chromic anhydride. Finally, olefination of C-4, 5 and C-6, 7 was achieved by heating with excess DDQ in toluene followed by hydrogenation with 10% Pd-C. This then gave the final product taxamairin B.

Since the above synthesis was the only synthesis for this class of diterpenes it was decided that it would be a noteworthy side project to synthesis this type of diterpene by a simpler method. This method also uses a convergent approach by constructing an A ring precursor and a C ring precursor and then bringing the precursors together to form the B ring. The A ring precursor was synthesized by modifying a known method starting with 1, 3-dicyclohexanedione (**63**) (Shuzi et al., 1991). This diketone was dimethylated with methyl iodide and K_2CO_3 in refluxing acetone in give a yield of about 65% after vacuum distillation. The 2, 2-dimethyl-1, 3-cyclohexanedione product (**64**) was then mono-

49 + 56

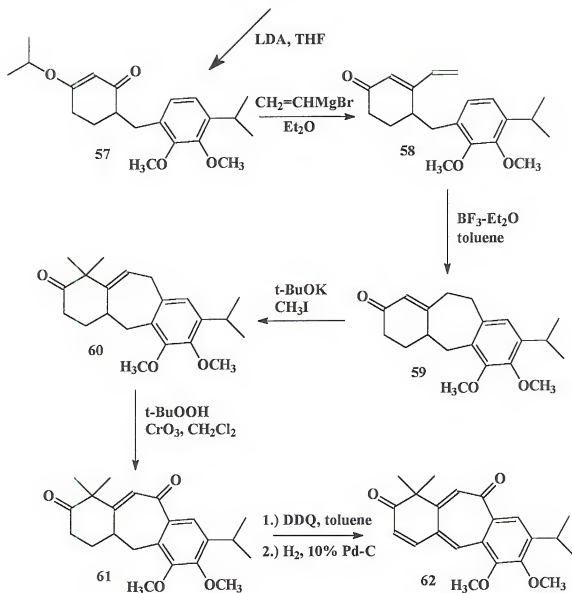


Figure 2-10: Literature Synthesis of Taxamairin B

brominated by slowly adding Br₂ in dichloromethane at room temperature while closely monitoring the TLC. This process gave a yield of 60% after purifying the product by column chromatography. Finally, 4-bromo-2, 2-dimethyl-1, 3-cyclohexanedione (**65**) was

dehydrohalogenated by refluxing with excess LiCl in DMF for 2 hours. This process gave a yield of 82% after purification by column chromatography of the A ring precursor 2, 2-dimethyl-4-cyclohexene-1, 3-dione (**66**) (Figure 2-11).

The formation of the C ring precursor was more problematic. Acetovanillone (**67**) was used as the starting material and this compound was isopropylated by heating with 90% H₂SO₄ and isopropyl alcohol at 60° C for 36 hours. Unfortunately this reaction could not be pushed beyond 50% conversion based on TLC of the reaction mixture, and after purification by column chromatography gave a yield of 40%. However this process is a better alternative than the 5-step process outlined in the previous synthesis for placing the isopropyl group on the ring. Following this, the phenolic group was methylated using dimethylsulfate and K₂CO₃ in refluxing acetone for 2 hours. This process was nearly quantitative to yield the dimethoxy product (**68**) (Figure 2-11). At this point a one carbon oxygenated substituent had to be introduced between the acetyl and methoxyl groups. Initially, a Vilsmeier-Haack reaction was attempted, but this gave a variety of products in which the acetyl seemed to undergo some reaction; however, no effort was made to characterize these products. Undesirable reactions also occurred with this method if the acetyl was first reduced to an alcohol group or completely reduced to an ethyl group. Attempts were also made to acetoxymethylate the desired position so the resulting acetate could be hydrolyzed and the alcohol oxidized to the aldehyde. The reaction was performed using 85% H₃PO₄, acetic anhydride, and paraformaldehyde and the chosen substrate was the reduced ethyl compound (**73**) which has less steric bulk and is more activated than the

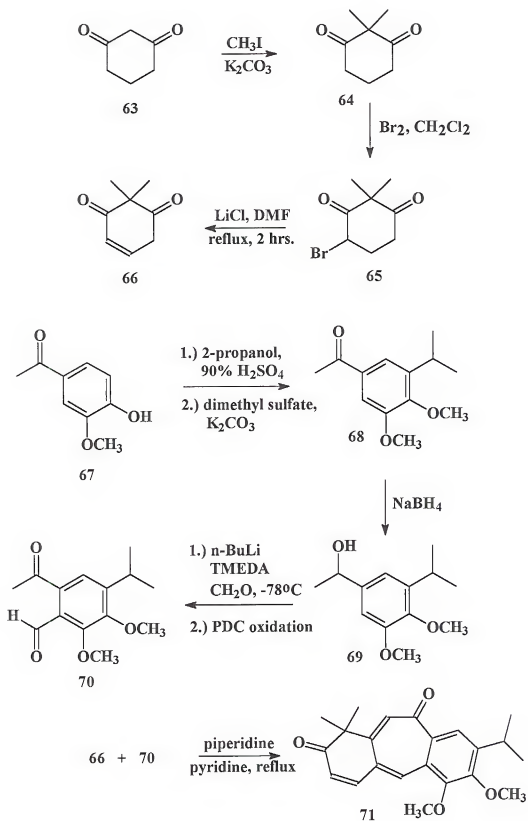


Figure 2-11: Synthesis of Taxamairin B

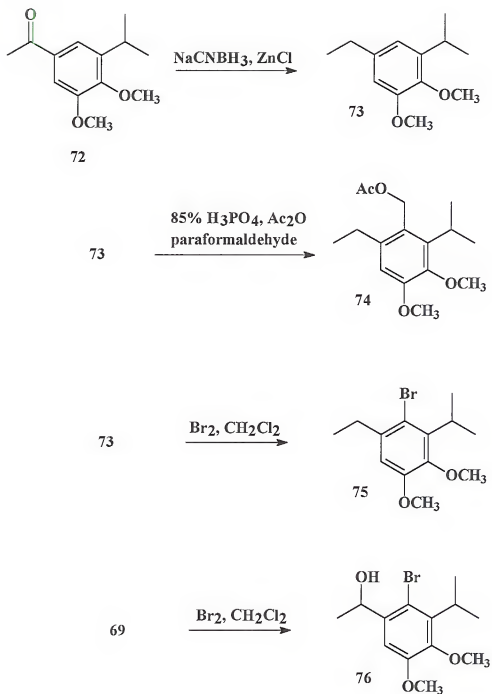


Figure 2-12: Reactions of Wrong Regioselectivity

oxygenated analogues. This substrate was obtained by reducing ketone **72** with NaCNBH_3 in the presence of ZnCl_2 (Figure 2-12). Although acetoxymethylation proceeded quite well the regioselectivity was wrong as was determined by NOE experiments (Figure 2-12). These experiments clearly showed enhancement of one of the methoxy methyls when the aromatic proton was irradiated; likewise when the oxygenated methylene was irradiated the isopropyl methyne signal was enhanced.

At this point lithiation of the aromatic ring and reaction with paraformaldehyde seemed to be a more attractive way of introducing a hydroxymethyl group which could then be oxidized to the aldehyde. Initially, lithium/halogen exchange was the desired process but bromination of the reduced alcohol substrate (**69**) as well as the totally reduced ethyl substrate (**73**) yielded a brominated product with the wrong regiochemistry (**75**, **76**) (Figure 2-12). This conclusion was reached following NOE experiments as previously described above.

In light of these results a direct lithiation was attempted with *n*-butyllithium in diethyl ether and TMEDA at -78°C using the reduced alcohol substrate (**69**) (Figure 2-11). It is well known that lithium complexes with methoxy groups and therefore addition usually takes place ortho to a methoxy if one is present on the aromatic ring. About 30-45 minutes after adding the *n*-butyllithium, paraformaldehyde was added. This reaction proceeded smoothly however yields were only around 50%. In any event, the regioselectivity was as desired based on NOE with the hydroxymethyl group adding ortho to the methoxy.

The resulting diol was then oxidized to the keto-aldehyde (70) with the mild oxidizing reagent PDC. This product was coupled to 2, 2-dimethyl-4-cyclohexene-1, 3-dione (66) by a tandem cross-aldol reaction in pyridine and piperidine (Figure 2-11). Although other products were produced the desired product was the major one. This pathway was expected since the most acidic carbon would be adding to the most electrophilic carbon first. Once this occurs, the 7-membered ring would be expected to close rather easily by another aldol reaction. The minor products were undoubtedly a variety of other cross-aldol products. This major product matched taxamairin B, which was obtained by methylating taxamairin A, in every way.

Isolation of Minor Compounds from *Taxus floridana*

The same reverse-phase chromatography protocol previously described was applied to the needles of the Florida yew (*Taxus floridana*) with very good results (Rao et al., 1996a). Although there was some question initially if this protocol would work on needles as well as on bark because of the greater content of waxes and pigments found in the needles, these questions were put to rest as all of the lipophilic material remained on the column while using the appropriate taxane solvent system without clogging up the column. It was found that paclitaxel could be isolated from these needles in a yield of 0.01% and 10-deacetyl baccatin III could be obtained in yields of 0.06%. Again, by TLC analysis, this time of the pre-paclitaxel fractions, it was found that many unidentifiable compounds were present in the filtrates. These filtrates were combined and evaporated to

dryness and reapplied to a regular-phase silica column using dichloromethane with 0-10% acetone and then 10% acetone with 0-10% methanol in dichloromethane.

A few compounds eluted with straight dichloromethane and thus had to be run on another column. This work will be discussed later. Elution with 2% acetone/dichloromethane gave 1 β -hydroxy baccatin I (**35**, Figure 2-4) as mentioned before (Della Casa De Marcano & Halsall, 1970). This was followed by a compound that was earlier given the name taxiflorine (**77**). Taxiflorine is an example of an 11(15 \rightarrow 1)-abeotaxane meaning that the A ring has contracted to contain only 5 carbons. Taxiflorine was previously isolated by our group but was published with an incorrect structure assignment (**78**) (Rao et al., 1996a). Taxiflorine itself gives difficult to interpret ^1H and ^{13}C NMR spectra for reasons that will be discussed later; however, upon acetylation the spectra are easier to interpret. According to the original structural assignment (**78**), its acetate should be the same compound as 13-acetyl-13-decinnamoyl taxchinin B (**79**) previously isolated by another group (Das et al., 1995); however, the spectral properties of these two compounds did not match (Table 2-1). It was then postulated that the correct structure of taxiflorine is one in which the C-10 benzoate and C-9 hydroxy groups are reversed so that the hydroxyl is at the C-10 position. To confirm this idea taxiflorine was oxidized with Jones reagent to the ketone (**80**, Figure 2-14) and its ^{13}C spectrum was compared with those of some known C-9 and C-10 keto taxanes. The carbonyl signal of **80** seen at 192.2 ppm is consistent with an α , β -unsaturated ketone system, and in contrast to the 199-204 ppm signal of C-9 keto taxanes. Similarly, the C-12 signal of **80** is at 156.8

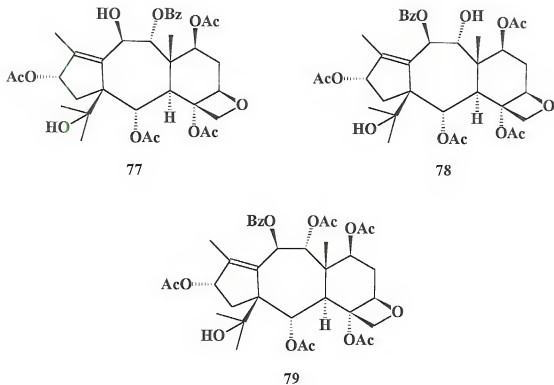


Figure 2-13: Structure of Taxiflorine and Related Compounds

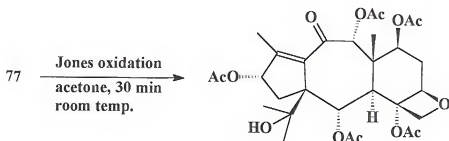


Figure 2-14: Oxidation of Taxiflorine

ppm which is also consistent with the β -carbon of an α , β -unsaturated ketone system. The C-12 signal in C-9 keto compounds is usually around 147.0 ppm. Also, the UV spectrum

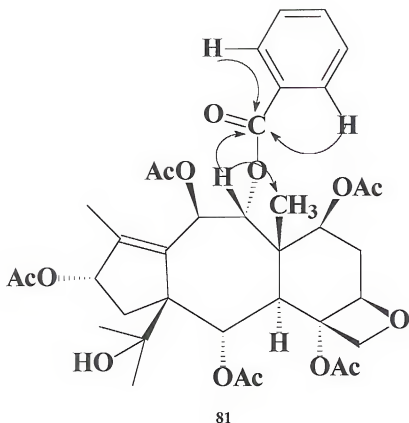


Figure 2-15: HMBC Correlation of Taxiflorine Acetate

of 80 displayed a λ_{max} at 232 nm with a shoulder at 253 nm, this shoulder is consistent with an α , β -unsaturated ketone system. Finally, to confirm the revised structure an HMBC spectrum was taken on the taxiflorine acetate (81, Figure 2-15). From this spectrum the interactions between the ortho protons and benzoate carbonyl carbon were clearly visible as was the interaction between the benzoate carbonyl carbon and the proton at C-9. Likewise the C-19 protons interacted with the C-9 carbon, which in turn interacted with the C-9 protons on the regular HETCOR spectrum. With this information in hand the

benzoate could be firmly placed at the C-9 position giving the correct structure of taxiflorine. This correcting structure was identical to a compound recently isolated from *Taxus chinensis* var. *Mairei* and given the name taxchinin M (Tanaka et al., 1996).

Elution with 5% acetone in dichloromethane yielded (-) rhododendrol (**82**, Figure 2-16) which has been reported previously in *Taxus brevifolia* (Chu et al., 1994) and *Betula pendula* (Smite et al., 1993). This was followed by 13-deacetyl taxiflorine (**83**, Figure 2-16). Like taxiflorine, **83** also exhibited a ^1H spectrum which contained very broad rounded peaks, whereas the spectrum of its acetate was normal. Also this acetate was identical to the acetate of taxiflorine. It was also discovered that it was possible to get a better spectrum of both these compounds (taxiflorine and 13-deacetyl taxiflorine) if the spectra were run at lower temperatures. Therefore ^1H and COSY spectra of **83** were

Table 2-1: ^1H and ^{13}C NMR Values for Related Abeo-Taxanes

H or C #	Compd. 81	Compd. 79	Compd. 80
1	****, 67.3	****, 68.5	****, 65.5
2	6.16 d (7.2Hz), 67.8	6.17 d (7.9Hz), 67.8	6.22 d (7.5Hz), 68.7
3	3.00 d (7.5Hz), 43.7	3.01 d (7.9Hz), 44.7	3.12 d (7.8Hz), 44.1
4	****, 78.6	****, 79.2	****, 79.0
5	4.96 d (7.2Hz), 84.6	4.99 d (7.6Hz), 84.6	5.00 d (6.0Hz), 84.9
6 α	2.67 m, 34.5	2.60 m, 34.7	2.74 m, 34.3
6 β	1.77 m, ****	1.91 m, ****	1.84 m, ****
7	5.54 t (7.8Hz), 70.2	5.59 t (8.2Hz), 70.6	5.16 t (7.5Hz), 71.0
8	****, 43.5	****, 43.5	****, 44.5
9	6.32 d (10.8), 77.2	6.21 d (10.9Hz), 76.3	6.32 s, 83.6
10	6.43 d (10.5), 67.6	6.58 d (10.9Hz), 68.8	****, 192.2
11	****, 135.8	****, 135.7	****, 137.5
12	****, 146.8	****, 147.7	****, 156.8
13	5.61 t (6.9Hz), 78.4	5.62 t (7.7Hz), 78.7	5.72 t (7.2Hz), 78.9
14 α	1.68 m, 36.6	2.50 m, 36.7	1.76 dd (8.1, 14.7Hz), 37.1

Table 2-1--continued

H or C #	Compd. 81	Compd.79	Compd.80
14 β	2.26 m, ****	2.00 m, ****	2.40 dd (7.2, 14.4Hz), ****
15	****, 75.2	****, 75.7	****, 76.3
16	1.17 s, 27.3	1.15 s, 27.7	1.22 s, 25.5
17	1.20 s, 24.9	1.07 s, 25.4	1.18 s, 27.4
18	1.87 s, 11.5	2.01 s, 11.9	2.08 s, 13.8
19	1.77 s, 13.0	1.68 s, 12.4	1.91 s, 13.6
20 α	4.49 d (7.2Hz), 74.3	4.50 d (7.7Hz), 74.5	4.56 d (7.2Hz), 74.7
20 β	4.41 d (7.2Hz), ****	4.41 d (7.7Hz), ****	4.45 d (7.2Hz), ****
q-Bz	****, 129.3	****, 129.0	****, 129.4
o-Bz	7.92 d (7.2Hz), 129.5	7.86 d (7.8Hz), 129.4	8.07 d (7.2Hz), 129.8
m-Bz	7.43 t (7.8Hz), 128.2	7.43 t (7.8Hz), 128.6	7.45 t (8.1Hz), 128.3
p-Bz	7.56 t (7.5Hz), 133.1	7.55 t (7.8Hz), 133.3	7.58 t (7.2Hz), 133.2
H or C #	Compd. 81	Compd. 79	Compd. 80
OCOCH ₃	1.65 s, 1.82 s, 2.03 s, 2.13 s, 2.14 s, 20.5, 21.0, 21.6, 21.7, 21.9	1.74 s, 2.02 s, 2.02 s, 2.08 s, 2.12 s, 20.6, 21.3, 21.4, 21.6, 22.0	2.01 s, 2.05 s, 2.11 s, 2.13 s, 20.9, 21.5, 21.6, 21.8
C=O	166.2, 167.8, 168.9, 169.7, 170.1, 170.3	163.9, 168.9, 169.0, 169.7, 170.2, 170.4	166.8, 169.0, 169.6, 170.3, 170.4

obtained at -10°C and the structure was determined to be that which is shown and which was previously isolated by another group from *Taxus chinensis* var. *Maiei* and given the name taxchinin L (Tanaka et al., 1996). It has been reported in the literature that abeotaxanes which contain a C-9 benzoate and a C-10 hydroxyl group usually give proton spectra in which the peaks are broad and rounded (Rao & Juchum, 1998). This is because

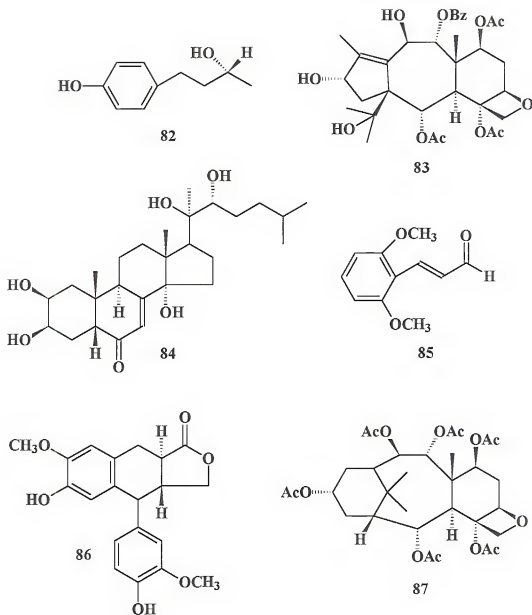


Figure 2-16: Compounds from the Needles of *Taxus floridana*

these compounds seem to exist in an equilibrium between two conformers. If the spectrum is taken at low temperature however, two sets of signals can be distinguished.

On elution with 10% acetone/dichloromethane additional amounts of 10-deacetyl baccatin III were obtained; and with 2% methanol/10% acetone/dichloromethane the polyhydroxylated steroid ponasterone A (**84**) was isolated (Figure 2-16). This compound was previously isolated from *Taxus brevifolia* (Rao et al., 1996b). Finally, with 5-10% methanol/10% acetone/dichloromethane 10-deacetyl paclitaxel-7- β -xyloside was isolated for the first time from *Taxus floridana*.

The fractions mentioned earlier that were eluted with dichloromethane were concentrated and put on another silica column using 25% ethyl acetate/ligroin as the starting mobile phase. At 30% ethyl acetate/ligroin trans-2,6-dimethoxy cinnamaldehyde (**85**) was eluted; and it will be discussed later. Elution continued with 50% ethyl acetate/ligroin, which gave the lignan α -conidendrin (**86**) followed by 1-deoxy baccatin IV (**87**); both of which have been previously isolated (Figure 2-16) (Miller et al., 1982; Miller, 1980).

Synthesis of Trans 2, 6-Dimethoxy Cinnamaldehyde

As mentioned above trans-2, 6-dimethoxy cinnamaldehyde was one of the compounds isolated from *Taxus floridana*. Although this structure was determined quite easily based on ^1H and ^{13}C NMR spectra, di-ortho oxy substituted $\text{C}_6\text{-C}_3$ compounds had previously not been isolated from natural products. Thus **85** was synthesized to verify the structure following Figure 2-17. Thus trans-2, 6-dimethoxy cinnamic acid (**88**) was methylated with methanol and H_2SO_4 followed by reduction to the alcohol (**89**) with LAH in a total yield of about 55%. The alcohol was then oxidized to the aldehyde (**90**) with

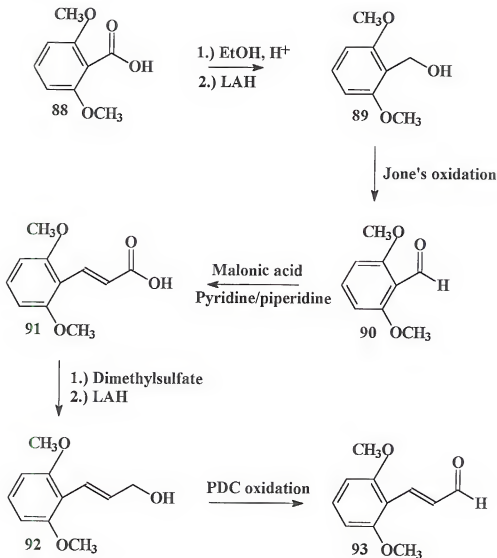


Figure 2-17: Synthesis of Trans 2,6-Dimethoxy Cinnamaldehyde

Jones reagent in a yield of 79% and the aldehyde was then condensed with malonic acid to yield the corresponding cinnamic acid (**91**) in about 85% yield. This acid was again methylated with dimethylsulfate and reduced with LAH to the alcohol (**92**) to give a total yield of 60%. Finally **92** was oxidized to the desired aldehyde (**93**) using the mild oxidizing agent PDC, to prevent further oxidation, in a yield of 38% (Figure 2-17). This aldehyde

was identical to the natural product in all ways. A thorough search of the literature confirmed that this was a novel compound and that no other 2, 6-dioxy cinnamyl compound has been found in nature.

Experimental

All reactions were monitored by silica gel 60 HF₂₅₄ TLC to ensure completion of the reaction. All NMR spectra were recorded using either a Varian VXR-300 or a Varian Gemini-300 spectrophotometer using CDCl₃ as solvent. Infrared spectra were obtained using a Perkin-Elmer 1420 ratio recording spectrophotometer. Ultraviolet spectra were obtained using a Shimadzu UV160U recording spectrophotometer. Mass spectra were recorded on a Finnigan Mat 950 Q spectrometer. Melting points were obtained by using a Fisher melting point apparatus. Column chromatography was accomplished using 100-200 mesh silica gel.

Isolation of Minor Compounds from *Taxus brevifolia*

The filtrates from the region between 10-deacetyl baccatin III and 10-deacetyl paclitaxel on the reverse-phase chromatographic separation were concentrated to a syrup (400 g). A 5 g aliquot was applied to a normal-phase silica column (100 g) in dichloromethane, and chromatographed with an elution sequence consisting of 1-5% acetone and then 5% acetone and 1-5% methanol. A total of 200 ml of each solvent mixture was used before progressing to the next solvent system and fractions of about 20 ml were collected and monitored by TLC. The order of elution was as follows: 1 β -

hydroxy baccatin I, baccatin VI, 1 β -hydroxy-7-deacetyl baccatin I, and 9-dihydro-13-acetyl baccatin III.

1 β -Hydroxy baccatin I (35)

The compound was eluted with 2% acetone in dichloromethane to give 281 mg of 35 crystallized from diethyl ether and ligroin. White crystalline powder, mp 259-261° C, ¹H NMR δ : 1.24 (s, 3H, 17-H), 1.25 (s, 3H, 19-H), 1.65 (s, 3H, 16-H), 1.80 (m, 1H, 6-H β), 1.90 (m, 1H, 14-H β), 2.00 (s, 3H, OAc), 2.05 (s, 3H, OAc), 2.06 (s, 3H, OAc), 2.06 (s, 3H, OAc), 2.09 (s, 3H, OAc), 2.12 (s, 3H, OAc), 2.18 (m, 1H, 6-H α), 2.22 (s, 3H, 18-H), 2.32 (d, 4.8Hz, 1H, 20-H β), 2.54 (dd, 9.9, 15.3Hz, 1H, 14-H α), 3.19 (d, 3.6Hz, 1H, 3-H), 3.56 (d, 5.4Hz, 1H, 20-H α), 4.22 (br s, 1H, 5-H), 5.49 (m, 2H, 2-H, 7-H), 6.05 (d, 11.1Hz, 1H, 9-H), 6.09 (t, 7.8Hz, 1H, 13-H), 6.22 (d, 11.4Hz, 1H, 10-H). ¹³C NMR δ : 13.6, 15.4, 20.6, 20.8, 20.9, 21.3, 21.4, 21.6, 21.8, 28.4, 31.0, 38.5, 41.3, 43.2, 46.6, 49.9, 58.3, 68.7, 70.7, 71.1, 72.1, 75.1, 76.0, 77.7, 135.6, 140.3, 169.0, 169.2, 169.3, 169.7, 169.8, 170.1.

Baccatin VI (36)

The compound was eluted with 4% acetone in dichloromethane to give 362 mg of 36 crystallized from diethyl ether and ligroin. White crystalline powder, mp 245-247° C, ¹H NMR δ : 1.23 (s, 3H, 17-H), 1.61 (s, 3H, 19-H), 1.79 (s, 3H, 16-H), 1.87 (m, 1H, 6-H β), 2.00 (s, 3H, OAc), 2.03 (s, 3H, 18-H), 2.10 (s, 3H, OAc), 2.11 (s, 3H, OAc), 2.17 (m, 2H, 14-H α , β), 2.20 (s, 3H, OAc), 2.29 (s, 3H, OAc), 2.51 (m, 1H, 6-H α), 3.18 (d, 5.7Hz, 1H, 3-H), 4.13 (d, 8.4Hz, 1H, 20-H β), 4.33 (d, 8.4Hz, 1H, 20-H α), 4.97 (d, 9.0Hz, 1H, 5-H), 5.87 (d, 6.0Hz, 1H, 2-H), 6.00 (d, 11.4Hz, 1H, 9-H), 6.17 (t, 8.1Hz,

1H, 13-H), 6.22 (d, 11.1Hz, 1H, 10-H), 7.48 (t, 7.8Hz, 2H, m-Bz), 7.61 (t, 7.2Hz, 1H, p-Bz), 8.10 (d, 7.2Hz, 2H, o-Bz). ¹³C NMR δ : 12.8, 14.9, 20.7, 20.9, 21.2, 21.4, 22.3, 22.7, 28.3, 34.5, 35.1, 42.8, 45.8, 47.3, 69.7, 70.4, 71.8, 73.3, 75.0, 76.4, 78.9, 81.5, 83.8, 128.6, 129.3, 130.1, 133.7, 135.6, 141.2, 166.9, 168.9, 169.1, 169.8, 170.1, 170.4.

1 β -Hydroxy-7-deacetyl baccatin I (37)

The compound was eluted with 4% acetone in dichloromethane to give 132 mg of 37 crystallized from diethyl ether and ligroin. White crystalline powder, mp 234-236° C, FABMS m/z: 611 (M + 1), ¹H NMR δ : 1.18 (s, 3H, 16-H), 1.24 (s, 3H, 19-H), 1.66 (s, 3H, 17-H), 1.85 (m, 2H, 14-H α , β), 1.92 (m, 2H, 6-H α , β), 2.04 (s, 3H, OAc), 2.05 (s, 3H, OAc), 2.11 (s, 3H, OAc), 2.13 (s, 3H, OAc), 2.14 (d, 1.2Hz, 3H, 18-H), 2.19 (s, 3H, OAc), 2.32 (d, 5.1Hz, 1H, 20-H β), 2.53 (dd, 9.6, 15.0Hz, 1H, 14-H α), 3.08 (d, 3.6Hz, 1H, 3-H), 3.52 (d, 5.1Hz, 1H, 20-H α), 4.21 (t, 3.0Hz, 1H, 5-H), 4.27 (dd, 4.8, 10.8, 1H, 7-H), 5.45 (d, 3.6Hz, 1H, 2-H), 6.07 (t, 7.2Hz, 1H, 13-H), 6.14 (d, 11.1Hz, 1H, 9-H), 6.20 (d, 10.8Hz, 1H, 10-H). ¹³C NMR δ : 12.5, 15.5, 20.5, 20.8, 20.9, 21.3, 21.6, 21.8, 28.5, 32.3, 38.4, 40.4, 43.3, 47.1, 49.9, 59.2, 69.1, 70.4, 71.8, 72.5, 76.1, 78.0, 78.1, 135.7, 140.7, 168.3, 169.1, 169.5, 169.6, 170.0.

9-Dihydro-13-acetyl baccatin III (38)

The compound was eluted with 5% acetone and 2% methanol in dichloromethane to give 184 mg of 38 crystallized from diethyl ether and ligroin. White crystalline powder, mp 243-244° C, FABMS m/z: 631 (M + 1), ¹H NMR δ : 1.25 (s, 3H, 16-H), 1.68 (s, 3H, 17-H), 1.82 (s, 3H, 19-H), 1.93 (d, 1.2Hz, 3H, 18-H), 1.96 (m, 1H, 6-H β), 2.14 (s, 3H, 10-OAc), 2.19 (s, 3H, 13-OAc), 2.21 (m, 2H, 14-H α , β), 2.28 (s, 3H, 4-OAc), 2.53 (m,

1H, 6-H α), 3.05 (d, 6.0Hz, 1H, 3-H), 4.16 (d, 8.1Hz, 1H, 20-H β), 4.31 (d, 8.1Hz, 1H, 20-H α), 4.43 (m, 2H, 7-H, 9-H), 4.95 (d, 8.4Hz, 1H, 5-H), 5.75 (d, 5.7Hz, 1H, 2-H), 6.17 (t, 6.9Hz, 1H, 13-H), 6.19 (d, 10.8, 1H, 10-H), 7.48 (t, 7.8Hz, 2H, m-Bz), 7.61 (t, 7.5Hz, 1H, p-Bz), 8.09 (d, 7.2Hz, 2H, o-Bz). ¹³C NMR δ : 12.5, 14.8, 21.2, 21.3, 22.6, 22.8, 28.3, 35.5, 38.0, 43.1, 45.0, 47.2, 69.8, 73.3, 73.7, 74.0, 76.9, 77.3, 78.8, 82.2, 84.1, 128.6, 129.4, 130.1, 133.6, 135.0, 139.7, 167.0, 169.3, 170.4.

Acetylation of 1 β -Hydroxy-7-Deacetyl Baccatin I

A total of 30 mg of 1 β -hydroxy-7-deacetyl baccatin I (37) was dissolved in 1 ml of acetic anhydride and 1 ml of pyridine. This mixture was stirred at room temperature for 18 hours and then water was added to the mixture. Sodium bicarbonate was added slowly until no further evolution of CO₂ was observed. The aqueous mixture was then extracted twice with dichloromethane and the combined organic layers were washed with 0.1 N NaOH, 0.1 N HCl, and water successively and dried with sodium sulfate. The dichloromethane was evaporated and the product was crystallized from diethyl ether and ligroin to yield 22 mg of acetylated 1 β -hydroxy-7-deacetyl baccatin I which was identical in every way to 1 β -hydroxy baccatin I (35).

Isolation of Taxamairin A (38) from *Taxus brevifolia*

A total of 27 g of semi-pure paclitaxel, which had crystallized from reverse-phase fractions, was dissolved in 200 ml of dichloromethane and applied to a silica column with 225 g of 240 mesh silica gel. Solvent was pumped through with an Eldex Laboratories metering pump model B-100-S-4 at a pressure not exceeding 25 psi. The beginning solvent was 2 : 1 dichloromethane : ligroin, then 3 : 1 dichloromethane : ligroin, followed

by dichloromethane. At this point the column was eluted with 2-5% acetone in dichloromethane and then 2-5% methanol and 5% acetone in dichloromethane. A total of 500 ml of each solvent mixture was pumped through before switching to the next solvent. Fractions of about 100 ml were collected and monitored by TLC. Taxamairin A was eluted with 2% acetone in dichloromethane and crystallized from the elution solvent. It was recrystallized from dichloromethane to yield 275 mg. Yellow needles, mp 252-253° C, EIMS m/z: 338 (80%, M), 310 (74%), 295 (100%), 267 (63%), 237 (18%), 156 (24%). CIMS: 339 (M + 1). UV λ_{max} : 211, 255, 385nm. IR (KBr): 1672, 1535, 1320, 1195, 1052 cm^{-1} . ^1H NMR δ : 1.33 (d, 6.9Hz, 6H, 19-H, 20-H), 1.46 (s, 6H, 12-H, 13-H), 3.35 (heptet, 6.9Hz, 1H, 18-H), 3.89 (s, 3H, 15-OMe), 6.11 (d, 9.6Hz, 1H, 7-H), 6.65 (s, 1H, 14-OH), 6.95 (s, 1H, 11-H), 7.31 (d, 9.9Hz, 1H, 11-H), 7.77 (s, 1H, 17-H), 7.94 (s, 1H, 4-H). ^{13}C NMR δ : 23.3, 23.4, 26.7, 26.8, 27.4, 50.5, 62.0, 119.3, 120.8, 123.7, 130.1, 131.1, 133.7, 136.6, 146.1, 146.8, 147.8, 148.2, 151.4, 188.2, 200.9.

Methylation of Taxamairin A

Taxamairin A (50 mg) was dissolved in 3 ml acetone and excess K_2CO_3 was added together with 0.25 ml of dimethyl sulfate. This mixture was refluxed for 3 hours and at that point 0.5 ml of concentrated NH_4OH was added to the mixture and stirred for 15 minutes. The acetone was partially evaporated and water was added. This aqueous solution was then extracted twice with dichloromethane and the combined organic layers were washed with 0.1 N NaOH and then with water. After drying with sodium sulfate, the solvent was removed and the residue was crystallized from dichloromethane to yield 32 mg of taxamairin B (39). Yellowish white needles, mp >290° C, UV λ_{max} : 219, 281, 355

nm. IR (KBr): 1670, 1621, 1333, 1305, 1038 cm^{-1} . ^1H NMR δ : 1.30 (d, 6.9Hz, 6H, 19-H, 20-H), 1.46 (s, 6H, 12-H, 13-H), 3.41 (heptet, 6.9Hz, 1H, 18-H), 3.98 (s, 6H, 14-OMe, 15-OMe), 6.12 (d, 9.6Hz, 1H, 7-H), 6.94 (s, 1H, 11-H), 7.31 (d, 1H, 11-H), 7.87 (s, 1H, 17-H), 7.93 (s, 1H, 4-H). ^{13}C NMR δ : 22.9, 23.0, 26.7, 26.8, 27.8, 50.4, 60.7, 61.2, 123.0, 124.1, 127.3, 128.3, 130.9, 131.5, 133.8, 136.1, 147.8, 150.8, 151.3, 153.9, 188.0, 200.7.

Synthesis of Taxamairin B (39)

2, 2-Dimethyl-1, 3-cyclohexanedione (64)

A total of 10 g of 1, 3-cyclohexanedione (63) and 30.6 g (2.5 eq.) of K_2CO_3 was added to 40 ml of acetone to which 31.5 g (2.5 eq.) of CH_3I had been added. The mixture was refluxed overnight. After cooling the mixture the K_2CO_3 was filtered and the acetone was removed under vacuum. The residual material was partitioned between water and diethyl ether and the water layer was discarded. The solvent was evaporated to yield a syrup which was poured into a mixture of 20 ml of conc. HCl and 20 g of ice. This was stirred for 30 minutes to decompose the methyl enol ether which accounts for about 30% of the mixture, then water and diethyl ether were added and partitioned. The organic layer was washed twice with water, then dried with sodium sulfate. After removal of the solvent, the crude liquid product was distilled using a water aspirator vacuum (~15 mm Hg) and the product distilled at 120-122° C. Upon standing the product crystallized yielding 6.2 g. Colorless crystals, mp 31-32° C, ^1H NMR δ : 1.29 (s, 6H, CH_3), 1.93 (m, 6.5Hz, 2H, 5-H), 2.67 (t, 6.9Hz, 4H, 4-H, 6-H). ^{13}C NMR δ : 18.1, 22.3, 37.4, 61.8, 210.6.

4-Bromo-2, 2-dimethyl-1, 3-cyclohexanedione (65)

A total of 2.0 g of dimethylated diketone **64** was dissolved in 5 ml of CH_2Cl_2 and a separate bromine mixture was prepared by adding excess bromine to CH_2Cl_2 in a ratio of about 4 drops bromine to 1 ml of CH_2Cl_2 . The bromine solution was dropwise added with stirring at room temperature to the diketone solution and the TLC was monitored using I_2 crystals as an indicator. The reaction was stopped when only a small amount of starting material was observed and the major spot on TLC had a slightly higher R_f value in 30% ethyl acetate in ligroin. Water was added to the reaction mixture and partitioned. The water layer was discarded and the organic layer was washed twice with water, dried with sodium sulfate, and the solvent was removed by evaporation. The residue was then put on a silica column using 20-30% ethyl acetate as the solvent to give 1.14 g of the product as a yellow oil. This material was stored at -5°C and upon storage the product crystallized. Colorless crystals, mp $48-50^\circ\text{C}$, ^1H NMR δ : 1.34 (s, 3H, CH_3), 1.45 (s, 3H, CH_3), 2.27-2.60 (m, 2H, 6-H), 2.72-2.97 (m, 2H, 5-H), 4.73 (dd, 4.2, 6.9Hz, 1H, 4-H). ^{13}C NMR δ : 23.1, 24.1, 26.5, 34.3, 48.8, 59.5, 203.2, 208.2.

2, 2-Dimethyl-4-cyclohexene-1, 3-dione (66)

A total of 1.0 g of bromo compound **65** was dissolved in 5 ml of DMF and 1.0 g of LiCl was also added. This mixture was refluxed for 2 hours at which time the TLC showed the presence of a slower moving product and only a small amount of starting material. Water and diethyl ether were added to the mixture and partitioned. The water layer was partitioned twice more with diethyl ether and all the organic layers were combined and washed with water twice, dried with sodium sulfate, and the solvent was

evaporated. The residue was ran through a silica column using 15-30% ethyl acetate in ligroin as the eluent to give 823 mg of product as a yellow oil. Yellow oil, ^1H NMR δ : 1.35 (s, 6H, CH_3), 3.35 (dd, 2.4, 3.9Hz, 2H, 6-H), 6.25 (dt, 2.1, 10.2Hz, 1H, 4-H), 7.03 (dt, 3.9, 10.2Hz, 1H, 5-H).

Isopropylation of acetovanillone

A total of 3.0 g of acetovanillone (67) was added to 20 ml of 90% H_2SO_4 and 2 ml of isopropyl alcohol was added. This mixture was stirred at 60° C for 36 hours. At this point about 50% conversion to the product was observed on TLC. Longer reaction times did not increase conversion and higher temperatures caused decomposition. Thus the mixture was diluted with ice water and diethyl ether and partitioned. The water layer was partitioned once again with ether and the ether layers combined. The organic layer was then partitioned twice with 1.0 N NaOH and the organic layer was discarded. The aqueous layer was acidified with concentrated HCl and extracted twice with diethyl ether. This organic layer was then washed with water twice, dried with sodium sulfate, and concentrated. The residue was chromatographed on a silica column using 20-40% ethyl acetate in ligroin as the eluent to give 1.42 g of product which crystallized on standing. Clear colorless crystals, mp 116-117° C, EIMS m/z : 208 (42%, M), 193 (100%), 163 (17%), 77 (12%), ^1H NMR δ : 1.27 (d, 6.9Hz, 6H, CH_3), 2.57 (s, 3H, CH_3), 3.33 (quintet, 6.9Hz, 1H, CH), 3.95 (s, 3H, OCH_3), 6.23 (br s, 1H, Ar-OH), 7.39 (d, 1.5Hz, 1H, Ar-H), 7.50 (d, 1.5Hz, 1H, Ar-H). ^{13}C NMR δ : 22.2, 26.2, 27.2, 56.2, 107.4, 121.0, 129.4, 133.7, 146.2, 147.6, 197.1.

Methylation of isopropyl acetovanillone

A total of 1.0 g of isopropylated acetovanillone was dissolved in 20 ml of acetone then 3.0 g of K_2CO_3 and 1 ml of dimethyl sulfate were added. The mixture was refluxed for 3 hours at which time no starting material remained according to TLC. Then 1 ml of conc. NH_4OH was added and the mixture was stirred for 30 minutes. The acetone was then partially removed and the residue was partitioned between water and diethyl ether. The organic layer was washed twice with water, dried with sodium sulfate, and concentrated to yield 956 mg of product (**68**) as a clear colorless oil.

Reduction of **68**

A total of 1.0 g of **68** was dissolved in 8 ml of methanol with 3 drops of 1.0 N NaOH. To this was added dropwise a solution of $NaBH_4$ in methanol with 1.0 N NaOH and the TLC was monitored. When no starting material remained the mixture was acidified with 0.1 N HCl and the methanol was partially removed. The residue was partitioned between water and diethyl ether and the organic layer was washed twice with water, dried with sodium sulfate, and concentrated under vacuum. This material was chromatographed on a silica column using 25-50% ethyl acetate in ligroin as eluent to give 856 mg of alcoholic product (**69**) as a clear colorless oil. Clear colorless oil, EIMS m/z: 224 (97%, M), 209 (100%), 179 (18%), 139 (90%), 124 (33%). 1H NMR δ : 1.21 (d, 6.9Hz, 6H, CH_3), 1.48 (d, 6.3Hz, 3H, CH_3), 3.34 (heptet, 6.6Hz, 1H, CH), 3.80 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 4.83 (q, 6.3Hz, 1H, CH), 6.81 (s, 2H, Ar-H). ^{13}C NMR δ : 23.4, 25.0, 26.8, 55.6, 60.8, 70.5, 106.6, 115.1, 141.6, 142.2, 145.3, 152.5.

Hydroxymethylation of 69

A total of 200 mg of **69** was dissolved in 3 ml of dry diethyl ether, 205 mg (2 eq.) of TMEDA was added, and the mixture was cooled to -78°C under a helium atmosphere. After cooling the mixture, 0.4 ml of 2.5 M n-butyllithium in hexanes (2.2 eq.) was added with stirring. After stirring for 30-45 minutes excess paraformaldehyde was added and the mixture was stirred overnight while warming to room temperature. The mixture was diluted with water and diethyl ether and partitioned. The organic layer was washed twice with water, dried with sodium sulfate, and concentrated. According to TLC about 50% of the product remained. This material was separated on a silica column using 30-50% ethyl acetate in ligroin as eluent and a total of 116 mg of product was isolated as a slightly yellow oil. Yellow oil, ^1H NMR δ : 1.22 (d, 6.9Hz, 6H, CH_3), 1.56 (d, 6.3Hz, 3H, CH_3), 3.32 (quintet, 6.9Hz, 1H, CH), 3.84 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 4.68 (d, 11.7Hz, 1H, CH_2O), 4.86 (d, 11.7Hz, 1H, CH_2O), 5.12 (q, 6.6, 12.9Hz, 1H, CH), 7.10 (s, 1H, Ar-H). ^{13}C NMR δ : 23.1, 23.4, 27.0, 33.5, 55.9, 60.6, 61.2, 67.2, 118.7, 129.9, 139.5, 142.6, 149.6, 151.5.

Oxidation of diol to keto-aldehyde **70**

A total of 600 mg of CrO_3 (1 eq.) was added to a solution of 950 mg of pyridine (2 eq.) in 15 ml of CH_2Cl_2 and this was stirred at room temperature for 15 minutes. At this point the PDC solution was added dropwise to a solution of 100 mg of the diol in 3 ml of CH_2Cl_2 . The TLC of the reaction mixture was always checked about 5 minutes after adding 3-4 drops of the PDC solution using 2, 4-dinitrophenylhydrazine as the indicator. More PDC was added until the reaction was complete according to TLC. At this point the

reaction was filtered and the filtrate was washed twice with 0.1 N HCl, twice with 0.1 N NaOH, and twice with water. The organic layer was then dried with Na_2SO_4 and evaporated under a vacuum to a solid residue. This material was separated on a regular silica column using 20-40% ethyl acetate in ligroin. A total of 62 mg of clear colorless oil was obtained. Clear colorless oil, ^1H NMR δ : 1.24 (d, 6.9Hz, 6H, CH_3), 2.49 (s, 3H, CH_3), 3.37 (m, 1H, CH), 3.91 (s, 3H, OCH_3), 3.95 (s, 3H, OCH_3), 7.08 (s, 1H, Ar-H), 10.33 (s, 1H, CHO).

Condensation of keto-aldehyde 70 with dione 66

A total of 100 mg of keto-aldehyde 70 was added to 3 ml of pyridine containing ~10 drops of piperidine. To this mixture was added 80 mg of dione 66 and the mixture was refluxed with stirring for 6 hours. At this time TLC analysis showed a product with the same R_f value as the methylated taxamairin A (taxamairin B) along with other products. The mixture was diluted with diethyl ether and washed three times with 0.1N HCl until the water layer was still acidic. The organic layer was then washed twice with water, dried with Na_2SO_4 , and evaporated under a vacuum to a solid residue. The product was isolated using a regular silica column with 20-40% ethyl acetate in ligroin and a total of 46 mg of product was isolated as a yellowish white amorphous solid. All spectral data was identical to that of the methylated taxamairin A.

Complete reduction of methyl isopropyl acetovanillone (72)

A total of 500 mg of methylated isopropyl acetovanillone was dissolved in 5 ml of THF and 1.0 g of ZnCl_2 was added and the mixture was stirred at 60° C. To this was added NaCNBH_3 in small portions and the TLC was monitored. When nearly all the

starting material had been converted to a faster moving product the mixture was diluted with water and diethyl ether and partitioned. The organic layer was washed with 0.1N HCl and twice with water, dried over sodium sulfate, and concentrated. The product was purified by silica chromatography using 15-20% ethyl acetate in ligroin as solvent. A total of 387 mg of product (**73**) was obtained as a slightly yellow oil. Yellow oil, ^1H NMR δ : 1.21 (d, 6.9Hz, 6H, CH_3), 1.24 (t, 7.5Hz, 3H, CH_3), 2.60 (q, 7.8, 15.3Hz, 3H, CH_3), 3.33 (quintet, 6.9Hz, 1H, CH), 3.79 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 6.60 (d, 1.5Hz, 1H, Ar-H), 6.67 (d, 1.5Hz, 1H, Ar-H). ^{13}C NMR δ : 15.6, 23.6, 26.7, 28.9, 55.6, 60.9, 109.3, 117.3, 140.0, 142.0, 144.1, 152.3.

Acetoxymethylation of **73**

A total of 100 mg of **73** was added to 2.0 ml of 85% H_3PO_4 and to this was added 200 mg of paraformaldehyde and 0.5 ml of acetic anhydride. The mixture was stirred at room temperature for 3 hours at which point the TLC showed most of the starting material to be gone and a slower moving product had formed. The mixture was partitioned between water and diethyl ether and the organic layer was washed twice with 0.1 N HCl and twice with water, dried with sodium sulfate, and concentrated. The residue was separated on a silica column using 15-25% ethyl acetate in ligroin as the solvent. A total of 64 mg of the product (**74**) was isolated as a clear colorless oil. Clear colorless oil, ^1H NMR δ : 1.21 (t, 7.5Hz, 3H, CH_3), 1.35 (d, 7.2Hz, 6H, CH_3), 2.07 (s, 3H, OAc), 2.68 (q, 7.5, 15.0Hz, 2H, CH_2), 3.22 (m, 1H, CH), 3.84 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 5.14 (s, 2H, OCH_2), 6.66 (s, 1H, Ar-H). ^{13}C NMR δ : 16.2, 21.0, 21.9, 26.8, 28.9, 55.5, 60.6, 60.7, 110.7, 122.9, 142.1, 146.6, 153.2, 171.2.

Bromination of 73

A total of 100 mg of **73** was dissolved in 3.0 ml of CH_2Cl_2 and a dilute bromine solution in CH_2Cl_2 was added dropwise with stirring at room temperature and the TLC was monitored. The addition was stopped when a small amount of starting material remained and a faster moving product spot was present. Water and more CH_2Cl_2 was added to the mixture and partitioned. The organic layer was washed twice with 0.1 N HCl and twice with water, dried with sodium sulfate, and concentrated. The residue was separated on a silica column using 15-25% ethyl acetate in ligroin and 73 mg of product (**75**) was isolated as a yellowish oil. Yellow oil, ^1H NMR δ : 1.22 (t, 7.5Hz, 3H, CH_3), 1.35 (d, 6.6Hz, 6H, CH_3), 2.75 (q, 7.5, 15.3Hz, 2H, CH_2), 3.72 (m, 1H, CH), 3.83 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 6.69 (s, 1H, Ar-H). ^{13}C NMR δ : 14.4, 21.0, 30.9, 34.2, 55.7, 60.9, 111.0, 139.0, 140.5, 152.0.

Bromination of 69

A total of 100 mg of **69** was dissolved in 3.0 ml of CH_2Cl_2 and a dilute bromine solution in CH_2Cl_2 was added dropwise with stirring at room temperature and the TLC was monitored. The addition was stopped when only a small amount of starting material remained and a faster moving product spot was present. Water and more CH_2Cl_2 was added to the mixture and partitioned. The organic layer was washed twice with 0.1 N HCl and twice with water, dried with sodium sulfate, and concentrated. The residue was separated on a silica column using 15-25% ethyl acetate in ligroin and 58 mg of product (**76**) was isolated as a yellowish oil. Yellow oil, ^1H NMR δ : 1.34 (t, 6.6Hz, 6H, CH_3), 2.02 (d, 6.9Hz, 3H, CH_3), 3.71 (m, 1H, CH), 3.86 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3),

5.78 (q, 6.6, 13.5Hz, 1H, CH), 7.10 (s, 1H, Ar-H). ^{13}C NMR δ : 20.9, 26.7, 35.4, 50.0, 55.8, 60.9, 109.8, 118.0, 140.6, 142.5, 152.5.

Isolation of Minor Compounds from *Taxus floridana*

The mother liquors of fractions that contained 10-deacetyl baccatin III from the reverse-phase column (25-40% acetonitrile in water) were concentrated to a syrup (10 g). On standing, this syrup became an amorphous solid and 3 g of this was applied to a normal-phase silica column (40 g) using dichloromethane as the starting solvent. Then the column was eluted successively with dichloromethane containing 2, 5, and 10% acetone and then with addition of 2, 5, and 10% methanol. A total of 200 ml of each solvent mixture was passed through the column before the next solvent mixture was started and 20 ml fractions were collected and monitored by TLC. The initial dichloromethane eluent was put aside for further chromatography and the order of elution of the more polar compounds was as follows: 1 β -hydroxy baccatin I, taxiflorine (taxchinin M), rhododendrol, taxchinin L, 10-deacetyl baccatin III, ponasterone A, and 10-deacetyl paclitaxel-7- β -xyloside.

The initial dichloromethane eluent (0.250 mg) was applied to another silica column (3 g) using 25% ethyl acetate in ligroin as the initial solvent and proceeding to 50% ethyl acetate in 10% intervals. A total of 50 ml of each solvent was used before progressing to the next solvent mixture and 5 ml fractions were collected and monitored by TLC. The order of elution was trans-2,6-dimethoxy cinnamaldehyde, α -conidendrin, and 1-deoxy baccatin IV.

1 β -Hydroxy baccatin I (35)

The compound was eluted with 2% acetone in dichloromethane and a total of 189 mg of **35** was crystallized from diethyl ether and ligroin. Its physical and spectral properties are identical to that reported above.

Taxiflorine (taxchinin M) (77)

The compound was eluted with 2% acetone in dichloromethane and a total of 129 mg was crystallized from diethyl ether and ligroin. No NMR was reported since the spectrum contained poorly defined peaks.

Rhododendrol (82)

This compound was eluted with 5% acetone in dichloromethane and a total 435 mg was crystallized directly from the eluting solvent. Clear colorless crystals, mp 74-76° C, ^1H NMR δ : 1.23 (d, 6.2Hz, 3H, 1-H), 1.73 (m, 2H, 3-H), 2.63 (m, 2H, 4-H), 3.83 (m, 1H, 2-H), 6.74 (m, 2H, m-Bz), 7.04 (m, 2H, o-Bz). ^{13}C NMR δ : 23.5, 31.2, 40.9, 67.7, 115.3, 129.4, 133.9, 153.8.

Taxchinin L (83)

This compound was eluted with 5% acetone in dichloromethane and a total of 162 mg was crystallized from diethyl ether and ligroin. White crystalline powder, mp 264-266° C. ^1H NMR (-10° C) δ : 1.02 (s, 3H, 16-H), 1.22 (s, 3H, 17-H), 1.43 (m, 1H, 14-H α), 1.73 (s, 3H, 19-H), 1.79 (s, 3H, 7-OAc), 1.81 (m, 1H, 6-H β), 1.96 (s, 3H, 18-H), 2.05 (s, 3H, 2-OAc), 2.13 (m, 1H, 14-H α), 2.17 (s, 3H, 4-OAc), 2.61 (m, 1H, 6-H α), 3.15 (d, 7.4Hz, 1H, 3-H), 4.45 (m, 2H, 13-H, 20-H β), 4.53 (d, 7.4Hz, 1H, 20-H α), 4.69 (t, 10.3Hz, 1H, 10-H), 4.94 (d, 6.2Hz, 1H, 5-H), 5.46 (dd, 5.6, 8.6Hz, 1H, 7-H), 5.95 (d,

7.3Hz, 1H, 2-H), 5.97 (d, 10.1Hz, 1H, 9-H), 7.45 (t, 7.5Hz, 2H, m-Bz), 7.57 (t, 6.9Hz, 1H, p-Bz), 7.99 (d, 7.5Hz, 2H, o-Bz). ^{13}C NMR (-10°C) δ : 11.3, 13.9, 21.6, 21.8, 22.4, 25.2, 27.2, 34.3, 39.2, 42.9, 43.4, 66.3, 66.7, 68.4, 69.9, 74.9, 76.2, 77.5, 79.3, 80.7, 85.3, 128.2, 129.7, 129.9, 133.0, 137.5, 146.0, 167.7, 170.7, 170.8, 171.6.

10-Deacetyl baccatin III (25)

This compound was eluted with 10% acetone in dichloromethane and a total of 112 mg was crystallized from diethyl ether and ligroin. Spectral properties were identical to those reported in the literature (Dennis et al., 1988).

Ponasterone A (84)

This compound was eluted with 10% acetone and 2% methanol in dichloromethane and a total of 83 mg was crystallized directly from the eluting solvent. Spectral properties were identical to those reported in the literature (Miller et al., 1982).

10-Deacetyl paclitaxel-7- β -xyloside (28)

This compound was eluted with 5% methanol and 10% acetone in dichloromethane and a total of 79 mg was obtained as an amorphous solid. Spectral properties were identical to those reported in the literature (Senilh et al., 1984).

Trans-2, 6-dimethoxy cinnamaldehyde (85)

This compound was eluted with 25% ethyl acetate in ligroin and a total of 27 mg was obtained as a glassy solid. UV λ_{max} : 313 nm. IR (KBr): 3100, 3000-2940, 2810, 2740-2700, 1660, 1605-1585, 1475, 1260, 1140, 1100-1080, 970, 840, 725 cm^{-1} . EIMS m/z : 192 (30%, M^+), 161 (100%), 149 (17%), 91 (15%). ^1H NMR δ : 3.90 (s, 6H, OMe), 6.58 (d, 8.4Hz, 2H, m-Ar), 7.17 (dd, 7.8, 16.0Hz, 1H, 2-H), 7.33 (t, 8.4Hz, 1H, p-

Ar), 7.93 (d, 16.0Hz, 1H, 3-H), 9.64 (d, 8.1Hz, 1H, 1-H). ^{13}C NMR δ : 55.8 x 2 (OMe), 103.6 (m-Ar), 112.1 (q-Ar), 131.6 (2-C), 132.6 (p-Ar), 144.5 (3-C), 160.5 (o-Ar), 196.4 (1-C).

α -Conidendrin (86)

This compound was eluted with 50% ethyl acetate in ligroin and a total of 38 mg was crystallized from diethyl ether and ligroin. White crystalline powder, mp 257-259° C, EIMS m/z : 356 (100%, M^+), 255 (13%), 241 (26%), 137 (14%). ^1H NMR δ : 2.5 (m, 2H), 2.7-3.1 (m, 1H), 3.73 (s, 3H), 3.78 (s, 3H), 3.9-4.2 (m, 4H), 6.26 (s, 1H), 6.53 (d, 2.0Hz, 1H), 6.58 (d, 8.0Hz, 1H), 6.62 (s, 1H), 6.73 (d, 8.0Hz, 1H). ^{13}C DEPT NMR δ : 29.3 (CH_2), 41.9 (CH), 47.5 (CH), 49.9 (CH), 55.9 (CH_3), 60.0 (CH_3), 71.9 (CH_2), 110.1 (CH), 111.3 (CH), 114.6 (CH), 115.1 (CH), 121.5 (CH), 126.3 (C), 131.7 (C), 134.0 (C), 144.2 (C), 144.9 (C), 145.5 (C), 147.0 (C), 177.0 (C).

1-Deoxy baccatin IV (87)

This compound was eluted with 50% ethyl acetate in ligroin and a total of 57 mg was crystallized from diethyl ether and ligroin. White crystalline powder, mp 262-264° C, ^1H NMR δ : 1.12 (s, 3H, 17-H), 1.54 (s, 3H, 19-H), 1.78 (m, 1H, 1-H), 1.79 (s, 3H, 16-H), 1.88 (m, 1H, 14-H β), 1.91 (m, 1H, 6-H β), 1.97 (s, 3H, 18-H), 2.02 (s, 3H, Oac), 2.05 (s, 3H, Oac), 2.08 (s, 3H, Oac), 2.10 (s, 3H, Oac), 2.16 (s, 3H, Oac), 2.17 (s, 3H, Oac), 2.41 (m, 1H, 14-H α), 2.50 (s, 1H, 6-H α), 2.87 (d, 1H, 3-H), 4.19 (d, 7.5Hz, 20-H β), 4.51 (d, 8.1Hz, 1H, 20-H α), 4.99 (d, 8.7Hz, 1H, 5-H), 5.52 (m, 2H, 2-H, 7-H), 5.91 (m, 2H, 9-H, 13-H), 6.15 (d, 11.4Hz, 1H, 10-H). ^{13}C NMR δ : 12.7, 14.9, 20.7, 20.8, 20.9,

21.2, 21.4, 21.6, 22.6, 26.9, 31.3, 34.6, 37.9, 44.5, 45.6, 46.9, 69.0, 71.0, 71.1, 71.9, 75.4, 77.2, 81.0, 83.9, 133.3, 138.9, 169.2, 169.3, 169.7, 170.1, 170.2, 170.4.

Acetylation of Taxiflorine

Taxiflorine (77) 120 mg was dissolved in 2 ml of acetic anhydride and 1 ml of pyridine was added. The mixture was stirred at room temperature for 18 hours and then water was added to the mixture. Sodium bicarbonate was added slowly until no further evolution of CO₂ was observed. The aqueous mixture was then extracted twice with dichloromethane and the combined organic layers were washed with 0.1 N NaOH, 0.1 N HCl, and water successively and dried with sodium sulfate. The dichloromethane was evaporated and the product (112 mg) (81) was obtained as a glassy solid. For NMR data see Table 2-1.

Oxidation of Taxiflorine

Taxiflorine (77) 50 mg was dissolved in 2 ml of acetone and a few drops of Jones reagent was added and the mixture was stirred at room temperature for 2 hours. At this time the acetone was partially evaporated and water was added. This aqueous mixture was extracted twice with dichloromethane and the combined organic layers were washed with 0.1 N NaOH and then with water and then dried with sodium sulfate. After the dichloromethane was evaporated the residue was crystallized from diethyl ether and ligroin to yield 36 mg of the ketone product. White crystalline powder. For NMR data see Table 2-1.

Acetylation of Taxchinin L (83)

Taxchinin L 100 mg was dissolved in 2 ml of acetic anhydride and 1 ml of pyridine was added. The mixture was stirred at room temperature for 18 hours and then water was added to the mixture. Sodium bicarbonate was added slowly until no further evolution of CO_2 was observed. The aqueous mixture was then extracted twice with dichloromethane and the combined organic layers were washed with 0.1 N NaOH, 0.1 N HCl, and water successively and dried with sodium sulfate. The dichloromethane was evaporated and the product (84 mg) was obtained as a glassy solid. The physical and spectral properties of this acetylated product (81) was identical with the acetate of taxiflorine in every way.

Synthesis of Trans-2, 6-Dimethoxycinnamaldehyde (85)**Methyl 2, 6-dimethoxybenzoate**

A total of 5.0 g of 2, 6-dimethoxybenzoic acid (88) was dissolved in 30 ml of methanol and 1.0 ml of concentrated H_2SO_4 was added. This mixture was refluxed for 48 hours at which time most of the methanol was removed under reduced pressure. The residue was partitioned between water and diethyl ether and the organic layer was partitioned with 0.1 N NaOH to remove the remaining starting material, this was performed three times. Finally the organic layer was washed with water twice and dried with sodium sulfate. Upon removal of the solvent under reduced pressure the methyl ester product crystallized to yield 3.1 g of product.

2, 6-Dimethoxybenzyl alcohol (89)

A total of 3.1 g of the methyl ester was dissolved in 15 ml of THF and this was cooled to 0°C . A total of 581 mg (1 eq.) of LAH was then carefully added to the mixture

with stirring. This mixture was then refluxed for 4 hours at which time no starting material remained. Thus about 10 ml of acetone was added to neutralize the remaining LAH and this was stirred for 18 hours. The solvent was then removed by rotovap and the residue was partitioned between water and diethyl ether. The organic layer was washed three times with water and was dried with sodium sulfate. Upon removal of the solvent by evaporation the product crystallized to yield 1.5 g of the product alcohol. Clear colorless crystals, ^1H NMR δ : 3.83 (s, 6H, OCH_3), 4.70 (s, 2H, CH_2), 6.46 (d, 8.4Hz, 2H, m-Ar), 7.13 (t, 8.7Hz, 1H, p-Ar).

2, 6-Dimethoxybenzaldehyde (90)

A total of 1.5 g of the alcohol (89) was dissolved in 10 ml of acetone and Jones reagent was added dropwise while the TLC was monitored using 2, 4-dinitrophenylhydrazine as the indicator. Quite suprisingly the aldehyde had a lower R_f value than the alcohol. The reaction was continued until no alcohol starting material was observed on TLC. The acetone was partially removed and the residue was partitioned between water and diethyl ether. The organic layer was washed with water twice and dried with sodium sulfate. Upon removal of the solvent under reduced pressure the product crystallized to yield 1.18 g of the aldehyde product. Clear colorless crystals, ^1H NMR δ : 3.85 (s, 6H, OCH_3), 6.42 (d, 8.4Hz, 2H, m-Ar), 7.33 (t, 8.6Hz, 1H, p-Ar), 10.35 (s, 1H, CHO).

Trans-2, 6-dimethoxycinnamic acid (91)

A total of 1.18 g of the aldehyde (90) and 1.38 g (2 eq.) of malonic acid was dissolved in 4 ml of pyridine and a few drops of piperidine were added. This mixture was

refluxed overnight. The following day the TLC seemed unchanged but 2, 4-dinitrophenyl hydrazine did not give a positive test indicating that no aldehyde remained and the cinnamic acid product had an identical R_f value. The mixture was partitioned between 0.1 N HCl and diethyl ether and the organic layer was washed twice more with 0.1 N HCl and then three times with water and dried with sodium sulfate. Upon evaporation of the solvent by evaporation 1.25 g of the cinnamic acid product crystallized.

Methyl trans-2, 6-dimethoxycinnamate

A total of 1.25 g of the cinnamic acid product (91) was dissolved in 15 ml of acetone and 3.0 g of K_2CO_3 was added with 0.5 ml of dimethyl sulfate. The mixture was refluxed for 3 hours at which time no starting material was observed by TLC and thus 2.0 ml of ammonium hydroxide was added to decompose any remaining dimethyl sulfate. After 15 minutes of stirring, the solvent was partially removed under vacuum and the residue was partitioned between water and diethyl ether. The organic layer was washed three times with water and then dried with sodium sulfate. Upon removal of the solvent 1.23 g of the methyl ester product crystallized.

Trans-2, 6-dimethoxycinnamyl alcohol (92)

A total of 1.23 g of the methyl ester was dissolved in 15 ml of THF and this was cooled to 0° C. A total of 223 mg (1 eq.) of LAH was then carefully added to the mixture with stirring. This mixture was then refluxed for 4 hours at which time no starting material remained so about 10 ml of acetone was added to neutralize the remaining LAH and this was stirred for 18 hours. The solvent was then removed by evaporation and the residue was partitioned between water and diethyl ether. The organic layer was washed three

times with water and was dried with sodium sulfate. Since minor impurities were also present in the mixture after workup a silica column was ran using 15-30% ethyl acetate in ligroin as the solvent. A total of 700 mg of the product alcohol was obtained as a clear yellowish liquid. ^1H NMR δ : 3.84 (s, 6H, OCH_3), 4.32 (d, 5.1Hz, 2H, 1-H), 6.56 (d, 8.4Hz, 2H, m-Ar), 6.75-6.92 (m, 2H, 2-H, 3-H), 7.15 (t, 8.1Hz, 1H, p-Ar). ^{13}C NMR δ : 55.6, 65.4, 103.8, 113.9, 121.7, 128.2, 132.6, 158.4.

Trans-2, 6-dimethoxycinnamaldehyde (93)

A total of 4.0 g of CrO_3 was dissolved in 6.5 ml of pyridine at 0°C and stirred until a reddish orange solid formed (PDC). At that point 561 mg of **92** was added in 1.0 ml of acetone and the reaction was stirred for 4 hours at room temperature. At this point the TLC showed only a small amount of starting material remaining so the reaction was stopped. All physical and chemical properties matched that of the natural product.

CHAPTER 3 PREPARATION OF NITRATE ESTERS OF PACLITAXEL AND RELATED TAXANES

Complete Nitration of Paclitaxel and Related Taxanes

In an attempt to nitrate the phenyl ring of the N-benzoyl phenylisoserine side chain in paclitaxel (Figure 3-1, 94) in order to determine its effect on potency, the compound was subjected to reaction with a 1 : 5 mixture of concentrated nitric acid in acetic anhydride and an equal volume of dichloromethane. The reaction proceeded readily at room temperature in 30 minutes to give a single product which exhibited a much higher R_f on TLC than paclitaxel and even higher than 2', 7-diacetyl paclitaxel (Mellado et al., 1984), thus eliminating the possibility that acid-catalyzed acetylation had occurred. The ^1H NMR spectrum showed a considerable downfield shift of the 2' and 7 proton signals (H-2' 4.78 ppm \rightarrow 5.69 ppm, H-7 4.40 ppm \rightarrow 5.75 ppm). Following characterization by elemental analysis and IR spectroscopy, which exhibited characteristic bands for nitrate esters at 1650, 1270, and 835 cm^{-1} , the structure was determined to be that of paclitaxel-2', 7-dinitrate ester (Figure 3-1, 95). After a review of the literature it was found that the conditions used are actually standard conditions for nitrate ester synthesis (Boschan et al., 1955). It was surprising that the product showed no evidence of rearrangement of the A-

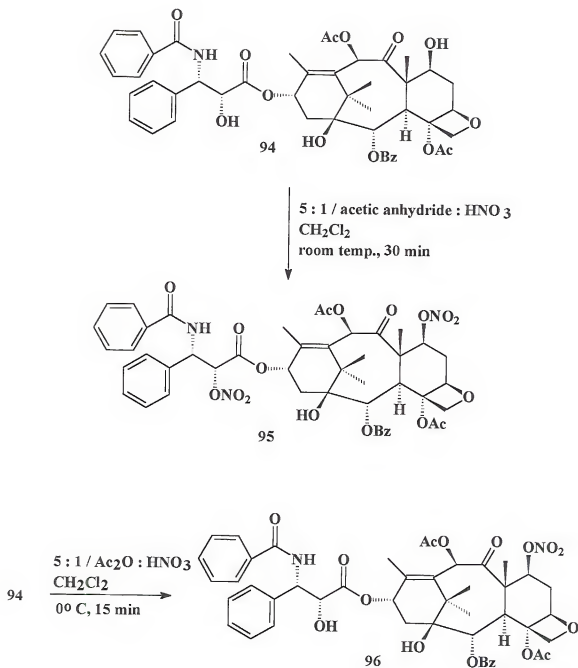


Figure 3-1: Nitration of Paclitaxel, 7-OH > 2'-OH

ring or cleavage of the oxetane ring, both of which have been known to occur in the presence of strong acids (Chen et al., 1993).

The reaction was also repeated with three of the natural analogues of paclitaxel, i.e., 10-deacetyl baccatin III, 10-deacetyl paclitaxel, and 10-deacetyl paclitaxel-7- β -xyloside. In each case the reaction proceeded to yield the corresponding tri- (Figure 3-2, 97), tri-(Figure 3-3, 101), and penta-nitrate esters (Figure 3-4, 104), respectively. All of these compounds crystallized readily from ethyl ether after workup without any need for chromatography to give nearly quantitative yields. In no case was nitration at the sterically hindered 1-hydroxyl observed. NMR chemical shift values are given in Table 3-1.

Table 3-1: ^1H and ^{13}C NMR Values for Completely Nitrated Taxanes

H or C#	Compd. 95	Compd. 97	Compd. 101	Compd. 104
1	****, 78.5	****, 78.2	****, 78.5	****, 78.7
2	5.72 d (6.9Hz), 74.3	5.64 d (6.6Hz), 73.5	5.71 d (6.9Hz), 74.0	5.71 d (6.9Hz), 74.5
3	4.02 d (6.9Hz), 47.2	3.92 d (6.9Hz), 47.7	3.95 d (6.9Hz), 47.2	3.81 d (6.6Hz), 45.7
4	****, 80.7	****, 80.2	****, 80.6	****, 80.6
5	4.99 d (9.0Hz), 83.6	4.97 d (8.7Hz), 83.5	4.98 d (9.0Hz), 83.4	4.88 d (9.0Hz), 83.5
6 α	2.69 m, 32.5	2.72 m, 32.5	2.71 m, 32.5	2.81 m, 35.8
6 β	2.04 m, ****	2.04 m, ****	2.07 m, ****	2.04 m, ****
7	5.75 dd (7.2, 10.5 Hz), 79.8	5.78 dd (7.2, 10.5 Hz), 79.9	5.75 dd (7.2, 10.5 Hz), 79.8	4.17 m, 80.1
8	****, 55.3	****, 55.8	****, 55.6	****, 57.8
9	****, 200.4	****, 199.3	****, 199.5	****, 199.9
10	6.31 s, 74.5	6.38 s, 81.4	6.36 s, 81.6	6.42 s, 82.1
11	****, 135.5	****, 134.2	****, 135.4	****, 135.5
12	****, 140.9	****, 142.2	****, 144.2	****, 143.1
13	6.30 t (9.9 Hz), 72.8	6.23 t (8.1 Hz), 78.1	6.29 t (9.0 Hz), 72.5	6.25 t (8.7 Hz), 72.6
14 α	2.38 m, 35.4	2.48 dd (9.9, 15.6 Hz), 34.2	2.39 m, 35.3	2.42 m, 35.3
14 β	2.38 m, ****	2.36 dd (2.8, 15.9 Hz), ****	2.39 m, ****	2.30 m, ****
15	****, 43.3	****, 42.9	****, 43.1	****, 42.9
16	1.23 s, 26.5	1.20 s, 26.6	1.20 s, 26.4	1.19 s, 26.2
17	1.17 s, 21.5	1.15 s, 20.3	1.14 s, 21.5	1.13 s, 21.6

Table 3-1--continued

H or C#	Compd. 95	Compd. 97	Compd. 101	Compd. 104
18	1.94 s, 14.3	2.12 s, 15.0	2.00 s, 14.8	1.97 s, 14.8
19	1.82 s, 11.0	1.82 s, 10.8	1.84 s, 11.0	1.75 s, 10.7
20 α	4.35 d (8.7 Hz), 76.2	4.36 d (8.7 Hz), 76.0	4.37 d (8.4 Hz), 76.2	4.35 d (8.7 Hz), 76.5
20 β	4.20 d (8.4 Hz), ****	4.11 d (8.4 Hz), ****	4.20 d (8.4 Hz), ****	4.19 d (8.7 Hz), ****
2'	5.69 d (3.0 Hz), 80.3	****	5.68 d (2.7 Hz), 80.2	5.65 d (3.0 Hz), 80.3
3'	6.14 dd (2.7, 9.6 Hz), 52.1	****	6.13 dd (2.7, 9.3 Hz), 52.1	6.10 dd (3.0, 9.3 Hz), 52.2
NH	6.97 d (9.6 Hz), ****	****	6.90 d (9.3 Hz), ****	6.82 d (9.3 Hz), ****
1''	****	****	****	4.76 d (4.5 Hz), 98.9
2''	****	****	****	4.98 dd (4.2, 6.3 Hz), 74.3
3''	****	****	****	5.26 t (6.3 Hz), 72.9
4''	****	****	****	5.08 dd (5.7, 9.6 Hz), 74.1
5'' ax	****	****	****	4.23 m, 59.5
5'' eq	****	****	****	3.68 dd (5.7, 12.9 Hz), ****
4-Ac	2.50 s, 22.6	2.43 s, 22.3	2.51 s, 22.6	2.48 s, 22.7
10-Ac	2.18 s, 20.6	****	****	****
OBz-1	****, 130.3	****, 128.6	****, 130.3	****, 129.0
OBz-2,6	8.12 d (7.2 Hz), 130.2	8.06 d (6.9 Hz), 130.1	8.13 d (7.5 Hz), 130.2	8.13 d (7.2 Hz), 130.2
OBz-3,5	7.52 t (7.8 Hz), 128.8	7.51 t (7.8 Hz), 128.9	7.52, 128.8	7.40-7.55, 128.8
OBz-4	7.63 t, 133.8	7.65 t, (7.5 Hz), 131.9	7.64 t (7.2 Hz), 133.9	7.63 t (6.6 Hz), 133.8
NBz-1	****, 131.2	****	****, 131.3	****, 131.2
NBz-2,6	7.73 d (7.2 Hz), 127.1	****	7.73 d (7.5 Hz), 127.1	7.73 d (6.9 Hz), 127.1
NBz-3,5	7.41-7.46, 129.4	****	7.41-7.46, 129.4	7.40-7.55, 129.4
NBz-4	7.41-7.46, 129.0	****	7.41-7.46, 129.1	7.40-7.55, 129.1
Ph-1	****, 133.1	****	****, 133.0	****, 133.1
Ph-2,6	7.41-7.46, 126.5	****	7.41-7.46, 126.5	7.40-7.55, 126.5
Ph-3,5	7.41-7.46, 128.8	****	7.41-7.46, 128.8	7.40-7.55, 128.8
Ph-4	7.49-7.54, 132.3	****	7.52, 132.4	7.40-7.55, 132.3

Table 3-3--continued

H or C#	Compd. 95	Compd. 97	Compd. 101	Compd. 104
C=O	170.2, 169.5, 167.7, 166.8, 166.7	171.1, 166.9	170.4, 167.6, 166.7, 166.7	170.3, 167.3, 166.8, 166.7

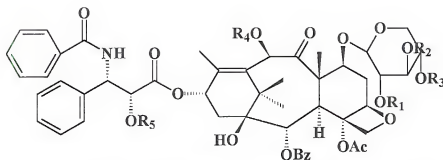
Regioselective Nitrations of Paclitaxel and Related Taxanes

Based on these results it was decided to run the reaction at 0° C for only 10-15 minutes to determine if the reaction was regioselective. Using this protocol, paclitaxel gave the 7-mononitrate of paclitaxel (**96**) in ~90% yield after crystallization from the crude reaction mixture (Figure 3-1). The position of the nitrate ester was easily determined by ¹H NMR and COSY spectroscopy as the nitrate ester causes a downfield shift of 1.3-1.7 ppm on the adjacent proton. This result was interesting since the 2'-hydroxyl is much more reactive than the 7-hydroxyl in acetylation reactions (Mellado et al., 1984). With nitration however the order of reactivity was 7-OH > 2'-OH.

Similar studies were applied to 10-deacetyl baccatin III, 10-deacetyl paclitaxel, and 10-deacetyl paclitaxel-7- β -xyloside. They also displayed some regioselectivity, however because these compounds contain more than 2 reactive hydroxyl groups, silica column chromatography was needed to separate the products. When 10-deacetyl baccatin III was subjected to this protocol three partially nitrated compounds were obtained namely the 10-mononitrate (**98**), the 10, 13-dinitrate (**99**), and the 7, 10-dinitrate (**100**), although **98** and **99** were the major compounds. From this it can be concluded that the order of reactivity is 10-OH > 13-OH > 7-OH. This also differs from the acetylation reactivities in which the 7-hydroxyl is the most reactive followed by the 10-hydroxyl and the 13-hydroxyl

respectively (Gueritte-Voegelein et al., 1986). The reaction of 10-deacetyl paclitaxel likewise gave the 10-mononitrate (**102**) and the 7, 10-dinitrate (**103**), again showing the 10-hydroxyl to be the most reactive and the 2'-hydroxyl the least ($10\text{-OH} > 7\text{-OH} > 2'\text{-OH}$). With acetylation the 2'-hydroxyl is the most reactive followed by the 7- and 10-hydroxyls respectively (Kingston et al., 1982). Finally, 10-deacetyl paclitaxel-7- β -xyloside was tested and because of the five available hydroxyls many products were observed on TLC and only the major products were isolated. These included the 2''-mononitrate (**105**), the 3''-mononitrate (**106**), the 4''-mononitrate (**107**), and the 2'', 3'', 4'', 10-tetranitrate (**108**) (Figure 3-2). This result indicates that the sugar hydroxyls are about equally reactive and more reactive than the 10-hydroxyl, with the 2'-hydroxyl being again the least reactive ($2'', 3'', 4'' > 10 > 2'$). Although no acetylation studies have been performed on the xylosides, this lab has shown that the 2'-hydroxyl is more reactive in standard acetylation conditions than either the sugar hydroxyls and the 10-hydroxyl is the least reactive (Figure 3-3).

At this point it should also be mentioned that the 10-deacetyl paclitaxel (**112**) used in this work was not isolated directly from biomass but was actually converted from 10-deacetyl paclitaxel-7- β -xyloside (**110**). This conversion involves oxidizing the xyloside to the dialdehyde (**111**) and then cleaving the dialdehyde with phenylhydrazine to give the desired product and the corresponding phenylhydrazones (Figure 3-4) (Rao, 1997). In conclusion, it has been shown that nitrate esters of taxanes can be formed under mild conditions and in many cases regioselectivity is shown. In view of this work it is



104 $R_1 = \text{NO}_2$, $R_2 = \text{NO}_2$, $R_3 = \text{NO}_2$, $R_4 = \text{NO}_2$, $R_5 = \text{NO}_2$

105 $R_1 = \text{NO}_2$, $R_2 = \text{H}$, $R_3 = \text{H}$, $R_4 = \text{H}$, $R_5 = \text{H}$

106 $R_1 = \text{H}$, $R_2 = \text{NO}_2$, $R_3 = \text{H}$, $R_4 = \text{H}$, $R_5 = \text{H}$

107 $R_1 = \text{H}$, $R_2 = \text{H}$, $R_3 = \text{NO}_2$, $R_4 = \text{H}$, $R_5 = \text{H}$

108 $R_1 = \text{NO}_2$, $R_2 = \text{NO}_2$, $R_3 = \text{NO}_2$, $R_4 = \text{NO}_2$, $R_5 = \text{H}$

Figure 3-2: Nitration of 10-Deacetyl Paclitaxel-7- β -Xyloside
 $1''\text{-OH}$, $2''\text{-OH}$, $3''\text{-OH}$ > 10-OH > $2'\text{-OH}$

10-Deacetyl Paclitaxel-7- β -Xyloside

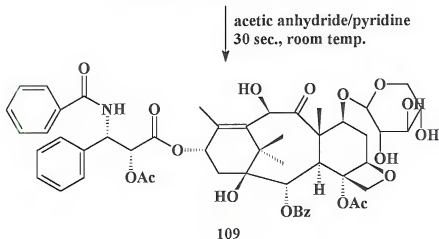


Figure 3-3: Regioselective Acetylation of
 10-Deacetyl Paclitaxel-7- β -Xyloside

conceivable that nitrate esters can be used as selective hydroxyl protecting groups when reactivity differing from acetylation reactivity is desired.

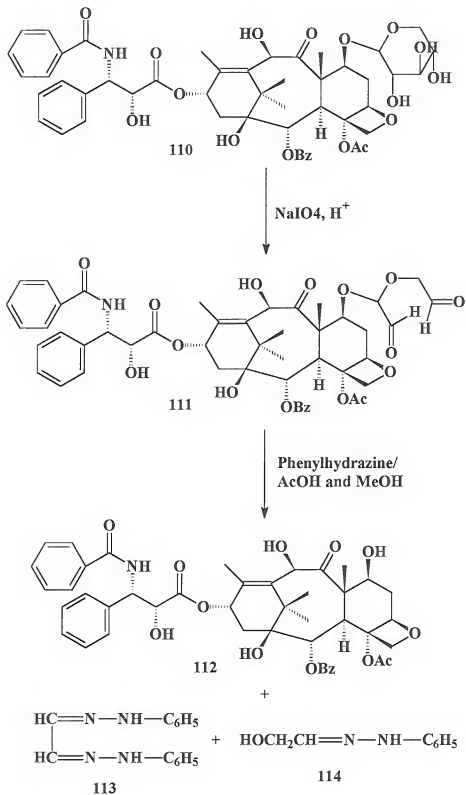


Figure 3-4: Conversion of 10-Deacetyl Paclitaxel Xyloside to 10-Deacetyl Paclitaxel

Reactions of Taxane Nitrate Esters

Complete Reductive Hydrolysis of Nitrate Esters with Zn and Acetic Acid

Since it had been shown that nitrate esters may serve as regioselective hydroxyl protecting groups, the next step was to determine under what conditions the nitrate esters may be removed without affecting the remainder of the molecule. Reductive nitration is known to occur under a variety of conditions and reagents including high-pressure catalytic hydrogenation, lithium aluminum hydride, hydrazine, Grignard reagents, metallic sodium, and hydrogen sulfide or ammonium sulfide (Green & Wuts, 1991). However many of these methods would undoubtedly react with the taxane structure also, therefore the method chosen was zinc in acetic acid. This method was not only very simple but also caused no rearrangements, hydrolysis, or any other side reactions. It involved dissolving the nitrate ester in acetic acid and adding zinc dust with stirring at room temperature for 30 minutes to give a quantitative yield of the parent alcohol (Figure 3-5).

At this point it was decided to run a series of reactions using a variety of reagents in order to determine what effect the conditions may have on the nitrate esters and/or taxanes. In all cases either paclitaxel-7, 2'-dinitrate or 10-deacetyl paclitaxel-7- β -xyloside-2'', 3'', 4'', 10, 2'-pentanitate was used in these reactions. A discussion of these reactions follows.

Reaction with NaBH_4

Paclitaxel-dinitrate was dissolved in methanol and an excess of NaBH_4 was added and stirred at room temperature. After 10 minutes TLC confirmed that the reaction was complete and two major products had formed. These products were determined by

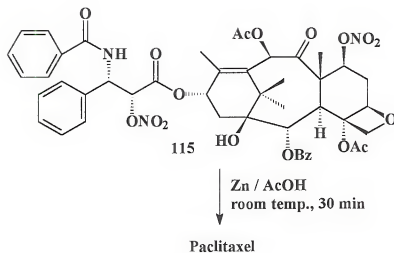
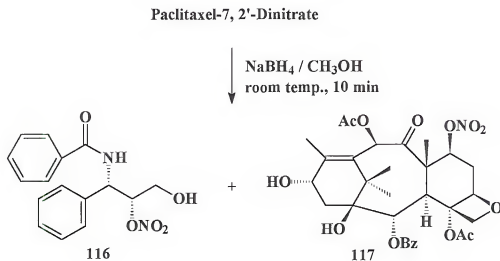


Figure 3-5: Reductive Denitration of Paclitaxel

Figure 3-6: Hydrolysis of the Side-Chain of Paclitaxel-7, 2'-Dinitrate with NaBH₄

NMR spectroscopy to be baccatin III-7-nitrate (**117**) and the side chain alcohol nitrate ester (**116**). Apparently the NaBH_4 serves only to quickly reduce the side chain ester (Figure 3-6).

Reaction with Ammonium Sulfide

Paclitaxel-dinitrate (**118**) was dissolved in acetonitrile and ammonium sulfide was added. After 2 minutes TLC showed a major slower moving product had formed. After isolation this product was determined to be paclitaxel-7-nitrate (**119**, Figure 3-7). This result indicates that the nitrate esters may be selectively removed at least under some conditions however this line of research was not studied further due to time constraints.

Acetylation of Taxane Nitrate Esters

In an effort to acetylate the 1-hydroxyl of 10-deacetyl paclitaxel-7-xyloside-pentanitate (**120**), this compound was dissolved in acetic anhydride and a small amount of DMAP was added. This was stirred at room temperature overnight. After workup the TLC of the reaction mixture displayed two major products (**121**, **122**) and essentially no starting material (Figure 3-8). After separation and isolation it was concluded by NMR spectroscopy that the faster moving product contained two additional acetates while the other product contained one additional acetate. FAB mass spectroscopy was used to determine that the molecular weight of the first compound was 1205 and that of the other was 1163, a difference of 42 or one acetate. The proton spectrum of **122** did not display any signal for H-2', H-3' or N-H. While the proton spectrum of **121** did not display any signal for H-2' or H-3', it did contain a far downfield signal at 11.41 ppm which could indicate a very acidic amide. In view of this information the structure of **121** and **122** were

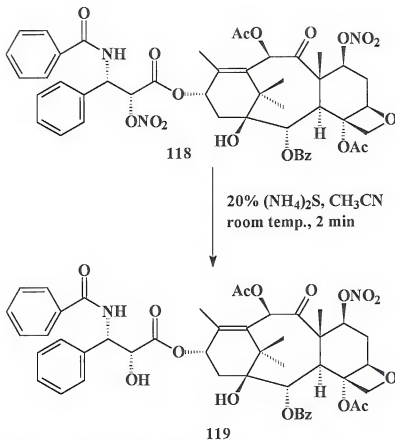


Figure 3-7: Regioselective Denitration of Paclitaxel-7, 2'-Dinitrate with Ammonium Sulfide

assigned as shown (Figure 3-8). Apparently, an enol acetate initially forms between the 2'- and 3'- positions to give compound 121. This can then form another enol-like acetate because of the increased acidity of the amide nitrogen to yield compound 122. At this point however it was not understood how the initial enol acetate was formed.

Reaction with NaN₃

In an attempt to displace a nitrate group, paclitaxel-dinitrate (123) was dissolved in acetonitrile and NaN₃ was added with stirring at room temperature. After two hours most of the starting material was no longer present and a product with similar R_f (124) was

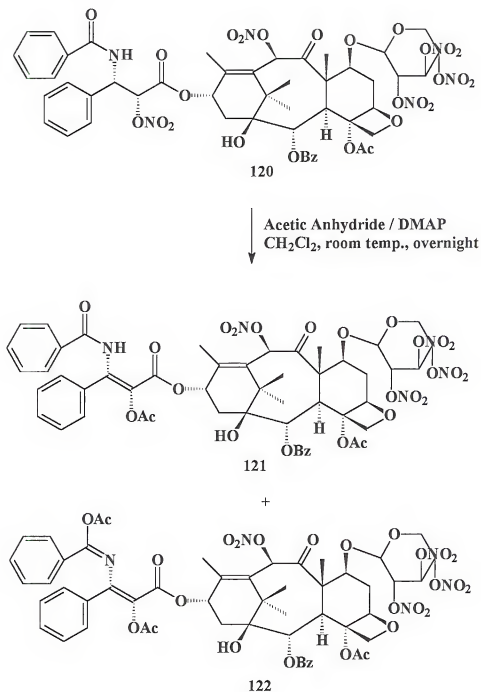


Figure 3-8: Enol Acetate Formation of Nitrate Esters

present on TLC (Figure 3-9). The reaction continued overnight and on the following day it was found that the initial product was no longer present and two other products (**125**, **126**) were now formed, one faster (**125**) than the "intermediate" product which exhibited strong UV absorbance but did not char with H_2SO_4 and a slower product (**126**) which exhibited UV absorbance as well as charring with acid on TLC. These two products were isolated by column chromatography and determined by NMR spectroscopy to be baccatin III-7-nitrate (slower product) (**126**) and dibenzamide (faster product) (**125**). Dibenzamide was also synthesized from benzamide and benzoyl chloride in the presence of NaH to insure that the reaction product was indeed dibenzamide. It was later found that in DMF as solvent the reaction proceeded much faster and if it were stopped after only 10 minutes the "intermediate" was the major constituent of the reaction mixture. This product was isolated and determined to have a molecular weight of 896. The ^1H NMR of this compound was very unusual in that many of the signals seemed to be in duplicate. Signals for the N-H and H-3' were present however there was no signal for the H-2'. The duplication of peaks was similar to what one may find in a racemic mixture presenting the possibility that the stereochemistry at one of the asymmetric carbons had been scrambled. After reviewing the literature concerning nitrate esters it was found that it is quite normal for a nitrate ester to undergo alpha-elimination in the presence of strong base to yield a carbonyl (Boschan et al., 1955). In this case the C-2' is already acidic due to its proximity to the C-1' ester carbonyl, therefore it was quite conceivable that even a weak base such as NaN_3 may cause alpha-elimination. With this information in hand the structure of **124** was determined to be as shown with the stereochemistry at C-3' racemic. It was also

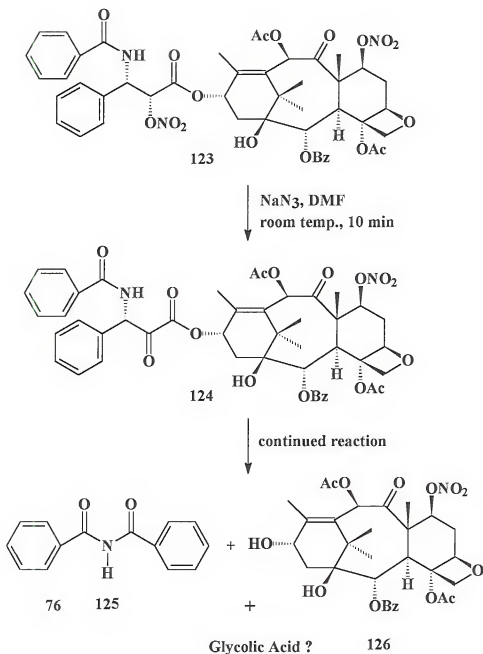


Figure 3-9: Reaction of 2'-Nitrate Ester with NaN_3

decided to confirm this structure by producing this compound by a more typical route.

Thus paclitaxel-7-nitrate (127) was oxidized with Jones reagent to yield a product (128)

possessing the same R_f value as the intermediate keto-ester (Figure 3-10). However this oxidation product only showed one set of signals on the ^1H NMR spectrum and this set of peaks matched one of the sets of peaks in the intermediate keto-ester ^1H NMR spectra. Presumably C-3' does not racemize in the acidic conditions of the Jones oxidation.

The formation of this keto-ester under basic conditions explains how the enol acetate and dienol acetate can form in the presence of acetic anhydride and pyridine (Figure 3-8). Once the keto-ester forms the H-3' can then be abstracted by the base to form the enolate which then undergoes O-acetylation and once the first enol acetate is formed the formation of the second proceeds as mentioned before. Indeed it was shown that if the paclitaxel keto-ester (**129**) was treated with pyridine in acetic anhydride the mono-enol acetate (**130**) was the major product (Figure 3-11). This compound was analogous to **121** (Figure 3-8) without the xylose. If this compound was reacted further under these same conditions a slightly faster moving product was formed that was probably the dienol acetate however it was not isolated due to time constraints. Also a second product was also obtained from the keto-ester acetylation that appears to be an enol acetate-enol. This conclusion was arrived at because unlike enol acetate **130** this compound does not show a down field N-H signal yet it has the same mass and the same number of acetates as **130**. On TLC however this compound has a much lower R_f than **130**, thus it is concluded that this compound may be either **131** or **132** (Figure 3-11).

One aspect of this that was not clear however was how the keto-ester breaks down under basic conditions to give dibenzamide and 10-deacetyl baccatin III-7-nitrate ester and what happens to the C-1' and C-2' carbons. It should be stated that this reaction proceeds

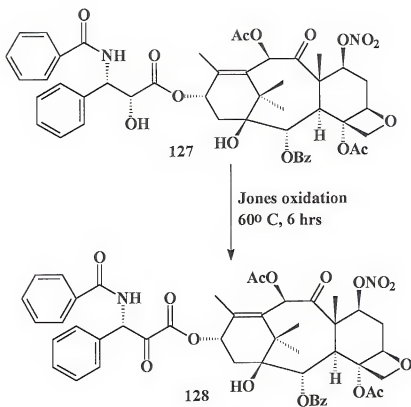


Figure 3-10: Oxidation of Paclitaxel-7-Nitrate Ester

in DMF or acetonitrile with all bases tried including NaN_3 , NaOAc , NaOBz , triethylamine, and hydroxide, with hydroxide giving the fastest reaction. However this reaction did not take place when using dichloromethane as solvent with NaN_3 . No other intermediate products were observed on TLC as loss of the intermediate keto-ester seemed to coincide with formation of the final products. In order to determine that this reaction was base catalyzed the intermediate keto-ester was subjected to three conditions; acetonitrile with dilute hydroxide added, neat acetonitrile, and acetonitrile with dilute HCl added. This study showed that after 24 hours the basic solution was 85-90% decomposed to the final

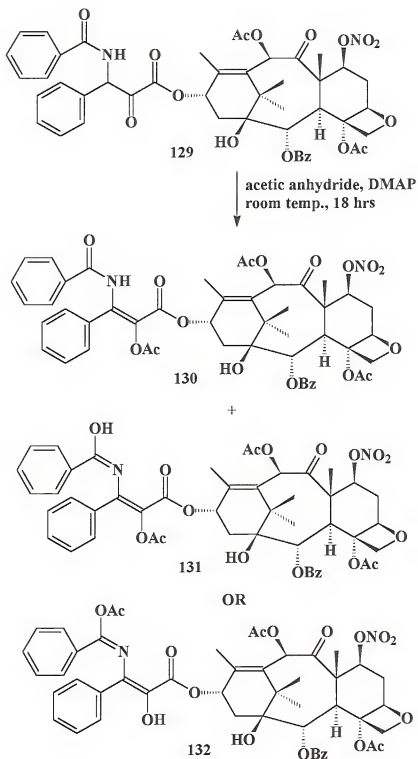


Figure 3-11: Acetylation of Keto-Ester

products, the neutral solution was about 40% decomposed, and the acid solution still contained almost all keto-ester.

Concerning the C-1' and C-2' fragment, it was assumed that C-1' exist as a carboxyl in its final form however it is unclear concerning the C-2'. Thus it can be concluded that this two carbon fragment may exist as acetic acid, glycolic acid, or glyoxalic acid in its final form. A failed attempt was made to derivatize the acid function by treating the reaction mixture with DCC and aniline to produce a UV active amide that could be isolated and characterized.

Unfortunately because of time constraints a mechanism for this rearrangement could not be conclusively established however a possible mechanism has been formulated and is presented below and in Figure 3-12. It has already been established that the keto-ester (133) can enolize in the presence of base to form the enolate and thus the enol (134). The amide proton in this enol is subsequently made quite acidic and can also be abstracted by base and after electron migration the imine alcohol can form (135). This conjugated imine is thus a reactive Michael-type adduct which can be attacked by hydroxide with the glycolic enolate serving as the leaving group. The imine can then be rearranged to form dibenzamide (138) while the C-1' ester is hydrolyzed giving glycolic acid (140) and baccatin III-7-nitrate ester (141). This hydrolysis has been shown to occur last because the ester enolate would presumably serve as a better leaving group than the acid enolate, however hydrolysis of the side chain may occur first. It would be interesting to test the hypothesis by subjecting the keto-acid to these conditions to see if the rearrangement still takes place. One could also alkylate the amide to a tertiary amide and determine if the

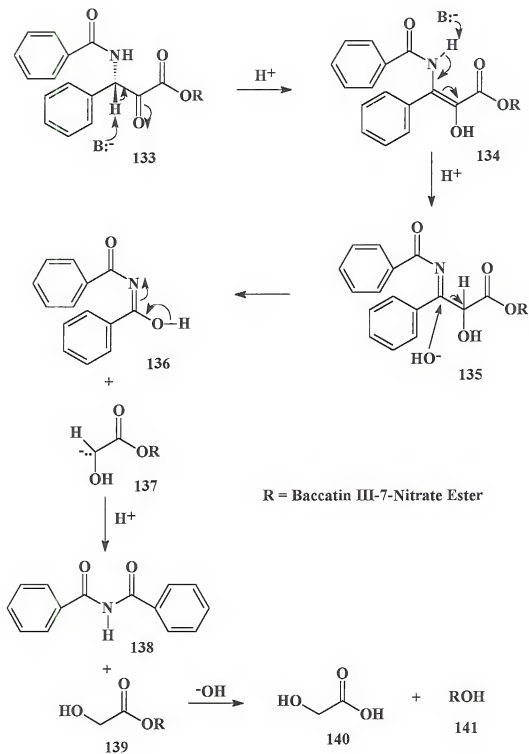


Figure 3-12: Mechanism of Keto-Ester Degradation

rearrangement takes place without this available amide proton. In any event time did not allow this to be studied further.

Experimental

All reactions were monitored by silica gel 60 HF₂₅₄ TLC to ensure completion of the reaction. All NMR spectra were recorded using either a Varian VXR-300 or a Varian Gemini-300 spectrophotometer using CDCl₃ as solvent. Infrared spectra were obtained using a Perkin-Elmer 1420 ratio recording spectrophotometer. Ultraviolet spectra were obtained using a Shimadzu UV160U recording spectrophotometer. Mass spectra were recorded on a Finnigan Mat 950 Q spectrometer. Melting points were obtained by using a Fisher melting point apparatus. Column chromatography was used in conjunction with 100-200 mesh silica gel.

Complete Nitrations of Taxanes

Paclitaxel-7, 2'-dinitrate ester (95)

Paclitaxel 500 mg was dissolved in 6 ml of CH₂Cl₂ and a mixture of 5 ml of acetic anhydride and 1 ml of concentrated nitric acid was added slowly. The mixture was prepared by slowly adding the nitric acid to ice cold acetic anhydride so that the mixture does not get too hot. The reaction mixture was allowed to stir at room temperature for 30 minutes. At this point 20 ml of water was added and while stirring NaHCO₃ was slowly added until no further frothing was observed. Additional CH₂Cl₂ was added and the water layer was extracted 3 times with CH₂Cl₂. The organic layer was dried with Na₂SO₄ and the solvent was evaporated. The product was crystallized with diethyl ether and ligroin to

yield 492 mg. White crystalline powder, mp 166-168° C, IR (KBr) 3450, 1725, 1650, 1365, 1270, 1230, 1065, 835, 700 cm^{-1} , Anal. Calc. for $\text{C}_{47}\text{H}_{51}\text{N}_3\text{O}_{18}$: C 58.69; H 5.31; N 4.37. Fd. C 58.83, H 5.15, N 4.11. ^1H and ^{13}C NMR see Table 1.

10-Deacetyl baccatin III-7, 10, 13-trinitrate ester (97)

This compound was prepared starting with 500 mg of 10-deacetyl baccatin III and following a procedure identical to that of paclitaxel-7, 2'-dinitrate. In this case however, the starting material does not initially dissolve in dichloromethane, but after reacting for a few minutes all material goes into solution. A total of 480 mg were crystallized from diethyl ether and ligroin. White crystalline powder, mp 159-161° C, Anal. Calc. for $\text{C}_{29}\text{H}_{33}\text{N}_3\text{O}_{16}$: C 51.25; H 4.90; N 6.18. Fd. C 51.63; H 5.25; N 5.83. ^1H and ^{13}C NMR see Table 1.

10-Deacetyl paclitaxel-7, 10, 2'-trinitrate ester (101)

This compound was prepared starting with 500 mg of 10-deacetyl paclitaxel and following a procedure identical to that of paclitaxel 7, 2'-dinitrate. A total of 506 mg of product was crystallized from diethyl ether and ligroin. White crystalline powder, mp 159-162° C, Anal. Calc. for $\text{C}_{43}\text{H}_{46}\text{N}_4\text{O}_{19} + \text{H}_2\text{O}$: C 56.02; H 5.01; N 5.81. Fd. C 56.07; H 4.91; N 5.64. ^1H and ^{13}C NMR see Table 1.

10-Deacetyl paclitaxel-7- β -xyloside-1'', 2'', 3'', 10, 2'-pentanitrates ester (104)

This compound was prepared starting with 500 mg of 10-deacetyl paclitaxel-7- β -xyloside and following a procedure identical to that of paclitaxel-7, 2'-dinitrate. As with 10-deacetyl baccatin III, all material went into solution only after the reaction had proceeded for a few minutes. A total of 512 mg of product was crystallized from diethyl

ether and ligroin. White crystalline powder, mp 187-188° C, Anal. Calc. for $C_{50}H_{52}N_6O_{27}$: C 51.38; H 4.48; N 7.19. Fd. C 51.53; H 4.59; N 6.82. 1H and ^{13}C NMR see Table 1.

Regioselective Nitration of Paclitaxel

Paclitaxel 500 mg was dissolved in 6 ml of dichloromethane and cooled to 0° C with an ice bath. A mixture of 5 ml of acetic anhydride and 1 ml of concentrated nitric acid also cooled to 0° C was added and the total was stirred in an ice bath for 15 minutes. At this point the reaction was worked up in the same manner as paclitaxel-7, 2'-dinitrate. Although a small amount of paclitaxel-7, 2'-dinitrate was seen on TLC, the product was sufficiently pure to be crystallized directly from diethyl ether and ligroin to give 435 mg of product (**96**). White crystalline powder, mp 163-165° C, Anal. Calc. for $C_{47}H_{50}N_2O_{16} + H_2O$: C 61.57; H 5.72; N 3.06. Fd. C 61.95; H 6.10; N 2.91. 1H NMR δ : 1.16 (s, 3H, 17-H), 1.22 (s, 3H, 16-H), 1.81 (s, 3H, 19-H), 1.83 (s, 3H, 18-H), 2.04 (m, 1H, 6-H β), 2.19 (s, 3H, 10-OAc), 2.36 (m, 2H, 14-H α,β), 2.41 (s, 3H, 4-OAc), 2.68 (m, 1H, 6-H α), 3.98 (d, 6.6Hz, 1H, 3-H), 4.18 (d, 8.4Hz, 1H, 20-H β), 4.33 (d, 8.7Hz, 1H, 20-H α), 4.81 (br s, 1H, 2'-H), 4.96 (d, 8.4Hz, 1H, 5-H), 5.68 (d, 6.9Hz, 1H, H-2), 5.74 (dd, 3.3, 10.5Hz, 1H, H-7), 5.79 (dd, 2.4, 9.0Hz, 1H, 3'-H), 6.21 (t, 8.1Hz, 1H, 13-H), 6.28 (s, 1H, 10-H), 7.10 (d, 9.0Hz, 1H, NH), 7.35-7.54 (m, 10H, m-Bz, o,m,p-Ph, m,p-NBz), 7.63 (t, 7.2Hz, 1H, p-Bz), 7.75 (d, 7.2Hz, 2H, o-NBz), 8.11 (d, 7.2Hz, 2H, o-Bz). ^{13}C NMR δ : 11.0, 14.5, 20.6, 21.1, 22.5, 26.6, 32.6, 35.6, 43.2, 47.3, 55.1, 55.3, 72.2, 73.1, 74.1, 74.8, 76.2, 78.5, 79.9, 80.6, 83.6, 127.0, 128.3, 128.7, 129.0, 130.1, 132.0, 133.0, 133.6, 133.8, 137.9, 141.0, 166.7, 167.3, 169.5, 170.7, 172.7, 200.4.

Regioselective Nitration of 10-Deacetyl Baccatin III

10-Deacetyl baccatin III 500 mg was dissolved in 6 ml of dichloromethane and cooled to 0° C with an ice bath. A mixture of 5 ml of acetic anhydride and 1 ml of concentrated nitric acid also cooled to 0° C was added and the total was stirred in an ice bath for 10 minutes. At this point the reaction was worked up in the same manner as paclitaxel-7, 2'-dinitrate. The TLC of the reaction mixture showed 4 compounds, two of which were a small amount of 10-deacetyl baccatin III (low R_f) starting material and a small amount of 10-deacetyl baccatin III-7, 10, 13-trinitrate (high R_f). A silica column was ran to isolate the two intermediate products using 0-15% acetone in dichloromethane as solvent. The more non-polar of the two products actually turned out to be a mixture of two compounds while the more polar product was determined by NMR spectroscopy to be 10-deacetyl baccatin III-10-mononitrate (**98**). The more non-polar two product mixture was then ran on another silica column using 30-50% ethyl acetate in ligroin as solvent, which separated the two compounds quite well. The product with the high R_f was determined to be 10-deacetyl baccatin III-10, 13-dinitrate (**99**), and the other to be 10-deacetyl baccatin III-7, 10-dinitrate (**100**). Product yields were as follows; 10-deacetyl baccatin III-10-mononitrate 180 mg crystallized from diethyl ether and ligroin, 10-deacetyl baccatin III-10, 13-dinitrate 115 mg crystallized from diethyl ether and ligroin, 10-deacetyl baccatin III-7, 10-dinitrate 62 mg.

10-Deacetyl baccatin III-10-mononitrate ester (98)

White crystalline powder, mp 169-171° C, Anal. Calc. for $C_{29}H_{33}NO_{12}$: C 59.08, H 5.98, N 2.38. Fd. C 58.90, H 6.28, N 2.19. 1H NMR δ : 1.09 (s, 3H, 17-H), 1.13 (s, 3H, 16-H), 1.70 (s, 3H, 19-H), 1.85 (m, 1H, 6-H β), 2.13 (s, 3H, 18-H), 2.16 (m, 2H, 14-H α,β), 2.30 (s, 3H, 4-OAc), 2.62 (m, 1H, 6-H α), 3.85 (d, 6.9Hz, 1H, 3-H), 4.15 (d, 8.4Hz, 1H, 20-H β), 4.32 (d, 8.1Hz, 1H, 20-H α), 4.41 (dd, 6.6, 10.2Hz, 1H, 7-H), 4.91 (t, 7.8Hz, 1H, 13-H), 4.97 (d, 9.6Hz, 1H, 5-H), 5.65 (d, 7.2Hz, 1H, 2-H), 6.49 (s, 1H, 10-H), 7.49 (t, 7.8Hz, 2H, m-Bz), 7.62 (t, 7.5Hz, 1H, p-Bz), 8.10 (d, 7.2Hz, 2H, o-Bz). ^{13}C NMR δ : 9.5, 15.8, 20.8, 22.5, 26.6, 36.6, 38.5, 42.5, 46.6, 58.5, 67.8, 71.6, 74.6, 76.5, 78.8, 80.7, 82.9, 84.1, 128.7, 129.2, 129.5, 130.1, 133.8, 148.3, 167.0, 170.8, 203.1.

10-Deacetyl baccatin III-10, 13-dinitrate ester (99)

White crystalline powder, mp 202-204° C, Anal. Calc. for $C_{29}H_{34}N_2O_{14}$: C 54.89, H 5.40, N 4.41. Fd. C 55.17, H 5.77, N 4.07. 1H NMR δ : 1.16 (s, 3H, 17-H), 1.18 (s, 3H, 16-H), 1.69 (s, 3H, 19-H), 1.87 (m, 1H, 6-H β), 2.08 (s, 3H, 18-H), 2.34 (m, 1H, 14-H β), 2.38 (s, 3H, 4-OAc), 2.46 (m, 1H, 14-H α), 2.60 (m, 1H, 6-H α), 3.77 (d, 7.2Hz, 1H, 3-H), 4.12 (d, 8.1Hz, 1H, 20-H β), 4.32 (d, 8.4Hz, 1H, 20-H α), 4.40 (dd, 6.6, 10.8Hz, 1H, 7-H), 4.94 (d, 9.0Hz, 1H, 5-H), 5.65 (d, 7.2Hz, 1H, 2-H), 6.21 (t, 8.1Hz, 1H, 13-H), 6.47 (s, 1H, 10-H), 7.50 (t, 8.1Hz, 2H, m-Bz), 7.64 (t, 7.2Hz, 1H, p-Bz), 8.06 (d, 7.2Hz, 2H, o-Bz). ^{13}C NMR δ : 9.4, 15.1, 20.6, 22.2, 26.5, 34.2, 36.6, 42.8, 46.6, 58.4, 60.4, 71.4, 74.2, 76.2, 78.2, 78.3, 80.7, 81.9, 84.1, 128.7, 128.8, 130.0, 132.4, 133.9, 141.6, 166.9, 170.1, 202.0.

10-Deacetyl baccatin III-7, 10-dinitrate ester (100)

White amorphous solid, Anal Calc. for $C_{29}H_{34}N_2O_{14}$: C 54.88; H 5.40; N 4.41. Fd. C 55.01; H 5.63; N 4.29. 1H NMR δ : 1.08 (s, 3H, 17-H), 1.10 (s, 3H, 16-H), 1.82 (s, 3H, 19-H), 2.05 (m, 1H, 6-H β), 2.16 (s, 3H, 18-H), 2.20 (m, 2H, 14-H α,β), 2.32 (s, 3H, 4-OAc), 2.72 (m, 1H, 6-H α), 3.99 (d, 6.9Hz, 1H, 3-H), 4.13 (d, 8.4Hz, 1H, 20-H β), 4.35 (d, 8.4Hz, 1H, 20-H α), 4.91 (t, 8.4Hz, 1H, 13-H), 4.99 (d, 9.0Hz, 1H, 5-H), 5.62 (d, 6.9Hz, 1H, 2-H), 5.80 (dd, 7.2, 10.5Hz, 1H, 7-H), 6.40 (s, 1H, 10-H), 7.50 (t, 7.5Hz, 2H, m-Bz), 7.63 (t, 7.5Hz, 1H, p-Bz), 8.09 (d, 7.2Hz, 2H, o-Bz). ^{13}C NMR δ : 10.8, 15.6, 20.4, 22.4, 26.6, 32.5, 38.4, 42.5, 47.8, 55.7, 60.4, 67.8, 73.8, 76.1, 78.5, 80.1, 80.2, 82.4, 83.5, 128.7, 128.9, 129.0, 130.0, 133.9, 148.9, 166.8, 171.1, 200.1.

Conversion of 10-Deacetyl Paclitaxel-7- β -Xyloside to 10-Deacetyl Paclitaxel (112)

10-Deacetyl paclitaxel-7- β -xyloside 1.0 g was dissolved in 10 ml of 1 : 1 THF and water and 2 ml of 1 N H_2SO_4 was added. This was followed by 0.71 g of $NaIO_4$ and the mixture was stirred overnight at room temperature. The mixture was diluted with water and extracted three times with dichloromethane. The organic layer was evaporated to dryness to yield 0.95 g of a white powder. The material was then dissolved in 20 ml of methanol and 0.5 ml of phenylhydrazine and 3 ml of acetic acid was added. This mixture was heated at 60° C for 3 hours at which point TLC analysis showed almost complete conversion to 10-deacetyl paclitaxel. The mixture was diluted with water, acidified and extracted with dichloromethane three times and the organic portion was evaporated to a dark red oil. The concentrate was partitioned in a countercurrent fashion, between 3 : 2 methanol/water and 4 : 1 benzene/ligroin as the two phases, and using 3 separatory funnels.

The benzene/ligroin layers which contained the phenylhydrazones were separated and concentrated to dryness. The combined methanol/water layer was concentrated partially and extracted three times with dichloromethane and the organic layer was concentrated to yield crude 10-deacetyl paclitaxel (0.8g). This material was clean enough for further work. All NMR spectra matched that of an authentic sample.

Regioselective Nitrations of 10-Deacetyl Paclitaxel

10-Deacetyl Paclitaxel 500 mg was dissolved in 6 ml dichloromethane and cooled to 0° C. Next 3 ml of a 5 : 1 mixture of acetic anhydride and concentrated nitric acid which was also cooled to 0° C was added and the reaction mixture was stirred in an ice bath for 5 minutes. The reaction was worked up as with paclitaxel-7, 2'-nitrate ester. Analysis of the reaction mixture by TLC revealed that practically all the starting material was gone and four products were present with two being major products. The fastest moving product was the completely nitrated 10-deacetyl paclitaxel and was in minor amounts. The next was a major product followed by two minor products and then by another major product. A silica column was used to separate this products with 0 → 15% acetone in dichloromethane as the solvent. From this column 175 mg of the faster major product (**103**) was obtained and crystallized from diethyl ether while 126 mg of the slower major product (**102**) was isolated but contained impurities. This was put on another column of the same type and solvent and 87 mg of the product was obtained. This material however would not crystallize. After analysis by NMR the faster product was determined to be 10-deacetyl paclitaxel-7, 10-dinitrate ester (**103**) and the slower product was the 10-mononitrate ester (**102**).

10-Deacetyl paclitaxel-10-mononitrate ester (102)

White amorphous powder, Anal. Calc. for $C_{45}H_{48}N_2O_{15}$: C 63.08; H 5.65; N 3.27. Fd. C 62.75; H 5.97; N 3.90. 1H NMR δ : 1.14 (s, 3H, 17-H), 1.19 (s, 3H, 16-H), 1.61 (s, 3H, 18-H), 1.83 (s, 3H, 18-H), 1.98 (m, 1H, 6-H β), 2.32 (m, 2H, 14-H α,β), 2.38 (s, 3H, 4-OAc), 2.56 (m, 1H, 6-H α), 3.75 (d, 6.9Hz, 1H, 3-H), 4.18, (d, 8.7Hz, 1H, 20-H β), 4.21 (dd, 3.6, 5.4Hz, 1H, 7-H), 4.30 (d, 8.1Hz, 1H, 20-H α), 4.79 (br s, 1H, 2'-H), 4.90 (d, 8.7Hz, 1H, 5-H), 5.68 (d, 7.2Hz, 1H, 2-H), 5.75 (dd, 2.1, 8.7Hz, 1H, 3'-H), 6.19 (t, 8.4Hz, 1H, 13-H), 6.41 (s, 1H, 10-H), 7.09 (d, 9.0Hz, 1H, NH), 7.35-7.53 (m, 10H, m-Bz, o,m,p-Ph, m,p-NBz), 7.61 (t, 7.2Hz, 1H, p-Bz), 7.73 (d, 6.9Hz, 2H, o-NBz), 8.11 (d, 7.2Hz, 2H, o-Bz). ^{13}C NMR δ : 9.6, 15.0, 21.6, 22.5, 26.4, 35.5, 36.6, 43.0, 46.2, 55.2, 58.4, 68.2, 71.5, 72.1, 73.1, 74.5, 76.5, 78.6, 81.0, 82.2, 84.1, 127.0, 127.1, 128.4, 128.7, 128.8, 129.0, 130.2, 130.7, 130.9, 132.0, 133.5, 133.8, 137.8, 143.8, 166.8, 167.3, 170.5, 172.6, 202.6.

10-Deacetyl paclitaxel-7, 10-dinitrate ester (103)

White crystalline powder, mp 170-172° C, Anal. Calc. for $C_{45}H_{47}N_3O_{18}$: C 58.76; H 5.37; N 4.57. Fd. C 59.11; H 5.27; N 4.49. 1H NMR δ : 1.13 (s, 3H, 17-H), 1.20 (s, 3H, 16-H), 1.83 (s, 3H, 19-H), 1.87 (s, 3H, 18-H), 2.06 (m, 1H, 6-H β), 2.36 (m, 2H, 14-H α,β), 2.41 (s, 3H, 4-OAc), 2.70 (m, 1H, 6-H α), 3.91 (d, 6.9Hz, 1H, 3-H), 4.17 (d, 8.4Hz, 1H, 20-H β), 4.34 (d, 8.4Hz, 1H, 20-H α), 4.80 (br s, 1H, 2'-H), 4.95 (d, 9.0Hz, 1H, 5-H), 5.67 (d, 6.9Hz, 1H, 2-H), 5.71 (dd, 7.2, 10.5Hz, 1H, 7-H), 5.77 (dd, 2.1, 8.7Hz, 1H, 3'-H), 6.20 (t, 9.0Hz, 1H, 13-H), 6.33 (s, 1H, 10-H), 7.07 (d, 9.0Hz, 1H, NH), 7.36-7.63 (m, 10-H, m-Bz, o,m,p-Ph, m,p-NBz), 7.63 (t, 7.5Hz, 1H, p-Bz), 7.73

(d, 7.2Hz, 2H, o-NBz), 8.10 (d, 6.9Hz, 2H, o-Bz). ^{13}C NMR δ : 10.9, 14.9, 21.2, 22.4, 26.4, 32.5, 35.5, 43.0, 47.3, 55.2, 55.6, 72.0, 73.1, 73.8, 76.2, 78.5, 79.9, 80.5, 81.7, 83.4, 127.0, 127.1, 128.4, 128.7, 128.8, 129.1, 130.1, 130.2, 131.2, 132.1, 133.5, 133.9, 137.7, 144.2, 166.7, 167.4, 170.9, 172.6, 199.5.

Regioselective Nitrations of 10-Deacetyl Paclitaxel-7- β -Xyloside

10-Deacetyl paclitaxel-7- β -xyloside 1.0 g was dissolved in 12 ml of dichloromethane and was cooled to 0° C. To this was added 6 ml of a 5 : 1 mixture of acetic anhydride and concentrated nitric acid which was also cooled to 0° C and the reaction mixture was stirred in an ice bath for 5-10 minutes. The reaction was worked up in the normal way and analyzed by TLC. This analysis showed many product spots however some seemed to be more predominate than others. Initially, a crude silica column was ran on this mixture using 0% methanol and 5% acetone in dichloromethane \rightarrow 5% methanol and 15% acetone in dichloromethane as the solvent. The fractions from this column were combined into three groups; fast, medium, and slow in elution order. The fast moving group contained some completely nitrated product, one major product that was slower than the completely nitrated one, and a couple of minor products. This material was ran on a silica column using 40% ethyl acetate in ligroin \rightarrow 60% ethyl acetate in ligroin as the solvent. A total of 116 mg of the major product was obtained and crystallized from dichloromethane. This product was determined to be 10-deacetyl paclitaxel-7- β -xyloside-2'', 3'', 4'', 10-tetranitrate ester (**108**). The middle group from the initial crude column contained many minor products and further separation was not attempted. The slowest group from the initial column contained three major products as

well as some starting material and minor products. A clean separation of the three major products was not possible using only one solvent system thus a first column was ran on this material using 40% → 80% ethyl acetate in ligroin. This eluted 84 mg of the slowest of the three products in pure form and this compound was determined to be 10-deacetyl paclitaxel-7- β -xyloside-2''-mononitrate ester (**105**). This product did not crystallize. The mixture of the remaining two products was separated on a silica column using 30% → 50% acetone in benzene as solvent. The faster of these two products was the 3''-mononitrate ester (**106**) and was crystallized from dichloromethane (76 mg). The slower product was determined to be the 4''-mononitrate ester (**107**) (101 mg) and was not crystallized.

10-Deacetyl paclitaxel-7- β -xyloside-2'', 3'', 4'', 10-tetranitrate ester (108**)**

White crystalline powder, mp 182-184° C, Anal. Calc. for C₅₀H₅₃N₅O₂₅: C 53.43, H 4.75; N 6.23. Fd. C 53.67; H 4.82; N 5.87. ¹H NMR δ : 1.12 (s, 3H, 17-H), 1.18 (s, 3H, 16-H), 1.75 (s, 3H, 19-H), 1.86 (s, 3H, 18-H), 2.03 (m, 1H, 6-H β), 2.34 (m, 2H, 14-H α , β), 2.39 (s, 3H, 4-OAc), 2.80 (m, 1H, 6-H α), 3.67 (dd, 5.4, 12.9Hz, 1H, 5''-Heq), 3.77 (d, 6.9Hz, 1H, 3-H), 4.14 (m, 1H, 5''-Hax), 4.20 (d, 8.1Hz, 1H, 20-H β), 4.21 (m, 1H, 7-H), 4.32 (d, 8.1Hz, 1H, 20-H α), 4.76 (d, 4.2Hz, 1H, 1''-H), 4.78 (d, 2.7Hz, 1H, 2'-H), 4.85 (d, 8.1Hz, 1H, 5-H), 4.97 (dd, 2.1, 3.9Hz, 1H, 2''-H), 5.07 (dd, 4.2, 5.4Hz, 1H, 4''-H), 5.26 (t, 6.0Hz, 1H, 3''-H), 5.69 (d, 6.9Hz, 1H, 2-H), 5.75 (dd, 2.1, 8.4Hz, 1H, 3'-H), 6.18 (t, 9.0Hz, 1H, 13-H), 6.39 (s, 1H, 10-H), 7.05 (d, 9.0Hz, 1H, N-H), 7.36-7.53 (m, 10H, m-Bz, o,m,p-Ph, m,p-NBz), 7.62 (t, 7.2Hz, 1H, p-Bz), 7.72 (d, 7.2Hz, 2H, o-NBz), 8.11 (d, 7.5Hz, 2H, o-Bz). ¹³C NMR δ : 10.6, 14.9, 21.4, 22.5, 26.2, 35.5, 35.8,

42.9, 45.7, 55.3, 57.8, 59.4, 72.0, 72.7, 73.2, 74.0, 74.2, 74.4, 76.4, 78.5, 80.2, 80.5, 82.2, 83.5, 98.8, 127.0, 127.1, 128.4, 128.7, 128.8, 129.0, 129.1, 130.1, 131.1, 132.1, 133.5, 133.8, 137.7, 143.2, 166.8, 167.4, 170.9, 172.7, 200.0.

10-Deacetyl paclitaxel-7- β -xyloside-2''-mononitrate ester (105)

White amorphous powder, Anal. Calc. for $C_{50}H_{56}N_2O_{19} + H_2O$: C 59.64; H 5.81; N 2.78. Fd. C 60.02; H 6.18; N 2.40. 1H NMR δ : 1.17 (s, 3H, 17-H), 1.26 (s, 3H, 16-H), 1.74 (s, 3H, 19-H), 1.81 (s, 3H, 18-H), 2.04 (m, 1H, 6-H β), 2.29 (m, 2H, 14-H α,β), 2.36 (s, 3H, 4-OAc), 2.72 (m, 1H, 6-H α), 3.17 (t, 10.8, 1H, 5''-Heq), 3.50 (t, 8.7Hz, 1H, 3''-H), 3.66 (dd, 7.5, 12.3Hz, 1H, 4''-H), 3.84 (d, 6.6Hz, 1H, 3-H), 3.91 (dd, 4.5, 11.4Hz, 1H, 5''-Hax), 4.04 (m, 1H, 7-H), 4.18 (m, 1H, 1''-H), 4.19 (d, 8.7Hz, 1H, 20-H β), 4.28 (d, 8.7Hz, 1H, 20-H α), 4.79 (m, 1H, 2'-H), 4.81 (m, 1H, 2''-H), 4.88 (d, 9.0Hz, 1H, 5-H), 5.07 (s, 1H, 10-H), 5.61 (d, 6.6Hz, 1H, 2-H), 5.73 (dd, 2.1, 8.7Hz, 1H, 3'-H), 6.15 (t, 7.8Hz, 1H, 13-H), 7.23 (d, 9.0Hz, 1H, N-H), 7.34-7.53 (m, 10H, m-Bz, o,m,p-Ph, m,p-NBz), 7.61 (t, 7.2Hz, 1H, p-Bz), 7.74 (d, 7.8Hz, 2H, o-NBz), 8.09 (d, 7.5Hz, 2H, o-Bz). ^{13}C NMR δ : 10.6, 14.2, 20.4, 22.5, 26.6, 35.7, 35.8, 43.0, 46.5, 55.3, 57.0, 65.1, 69.9, 72.4, 73.2, 73.6, 74.4, 74.6, 76.5, 78.6, 80.6, 80.9, 81.8, 84.0, 101.5, 127.1, 127.2, 128.4, 128.7, 128.8, 129.0, 129.1, 130.2, 132.1, 133.6, 133.7, 136.1, 137.8, 137.9, 166.8, 167.4, 170.8, 172.7, 209.2.

10-Deacetyl paclitaxel-7- β -xyloside-3''-mononitrate ester (106)

White crystalline powder, mp 209-211° C, Anal. Calc. for $C_{50}H_{56}N_2O_{19} + H_2O$: C 59.64; H 5.81; N 2.78. Fd. C 59.27; H 5.87; N 2.89. 1H NMR δ : 1.09 (s, 3H, 17-H), 1.19 (s, 3H, 16-H), 1.80 (s, 3H, 19-H), 1.81 (s, 3H, 18-H), 2.02 (m, 1H, 6-H β), 2.28 (m, 2H,

14-H α , β), 2.39 (s, 3H, 4-OAc), 2.71 (m, 1H, 6-H α), 3.35 (m, 1H, 5''-Heq), 3.38 (m, 1H, 2''-H), 3.75 (br s, 1H, 4''-H), 3.88 (d, 6.3Hz, 1H, 3-H), 3.95 (m, 1H, 5''-Hax), 4.08 (t, 8.1Hz, 1H, 7-H), 4.15 (d, 6.6Hz, 1H, 1''-H), 4.20 (d, 8.1Hz, 1H, 20-H β), 4.31 (d, 8.1Hz, 1H, 20-H α), 4.79 (br s, 1H, 2'-H), 4.90 (d, 9.0Hz, 1H, 5-H), 5.03 (t, 8.4Hz, 1H, 3''-H), 5.18 (s, 1H, 10-H), 5.65 (d, 6.6Hz, 1H, 2-H), 5.77 (d, 7.8Hz, 1H, 3'-H), 6.19 (t, 7.8Hz, 1H, 13-H), 7.15 (d, 7.5Hz, 1H, N-H), 7.40-7.53 (m, 10H, m-Bz, o,m,p-Ph, m,p-NBz), 7.62 (t, 6.9Hz, 1H, p-Bz), 7.75 (d, 7.5Hz, 2H, o-NBz), 8.12 (d, 7.2Hz, 2H, o-Bz). ¹³C NMR δ : 10.1, 13.6, 20.3, 22.0, 25.9, 34.9, 35.0, 42.6, 46.0, 55.1, 55.8, 65.0, 66.2, 69.6, 70.7, 73.4, 74.1, 74.3, 75.6, 78.5, 80.2, 81.3, 83.4, 86.2, 104.3, 126.4, 126.9, 127.7, 127.8, 127.9, 128.0, 129.3, 129.4, 130.8, 132.6, 133.8, 135.6, 137.0, 138.5, 165.6, 166.5, 169.6, 172.0, 208.5.

10-Deacetyl paclitaxel-7- β -xyloside-4''-mononitrate ester (107)

White crystalline powder, mp 193-195° C, Anal. Calc. for C₅₀H₅₆N₂O₁₉ + H₂O: C 59.64; H 5.81; N 2.78. Fd. C 59.96; H 6.18; N 2.56. ¹H NMR δ : 1.12 (s, 3H, 17-H), 1.25 (s, 3H, 16-H), 1.79 (s, 3H, 19-H), 1.81 (s, 3H, 18-H), 2.09 (m, 1H, 6-H β), 2.31 (m, 2H, 14-H α , β), 2.34 (s, 3H, 4-OAc), 2.64 (m, 1H, 6-H α), 3.01 (br s, 1H, 2''-H), 3.28 (t, 9.9Hz, 1H, 5''-Heq), 3.51 (t, 8.7Hz, 1H, 3''-H), 3.83 (d, 6.3Hz, 1H, 3-H), 3.98 (d, 7.5Hz, 1H, 5''-Hax), 4.11 (m, 1H, 1''-H), 4.12 (m, 1H, 7-H), 4.19 (d, 7.8Hz, 1H, 20-H β), 4.30 (d, 8.4Hz, 1H, 20-H α), 4.82 (br s, 1H, 2'-H), 4.90 (m, 1H, 5-H), 4.91 (m, 1H, 4''-H), 5.28 (s, 1H, 10-H), 5.64 (d, 6.6Hz, 1H, 2-H), 5.77 (d, 9.3Hz, 1H, 3'-H), 6.17 (t, 7.8Hz, 1H, 13-H), 7.21 (d, 9.3Hz, 1H, N-H), 7.31-7.53 (m, 10-H, m-Bz, o,m,p-Ph, m,p-NBz), 7.61 (t, 7.5Hz, 1H, p-Bz), 7.72 (d, 7.5Hz, 2H, o-NBz), 8.14 (d, 7.5Hz, 2H, o-Bz).

^{13}C NMR δ : 10.8, 14.2, 20.6, 22.6, 26.7, 35.3, 35.4, 43.1, 46.8, 54.9, 56.8, 61.6, 71.4, 72.4, 73.1, 74.5, 74.7, 76.6, 78.7, 79.7, 81.1, 81.7, 84.0, 104.4, 127.0, 127.1, 128.3, 128.7, 128.8, 128.9, 129.0, 130.3, 132.0, 133.6, 133.7, 136.1, 137.9, 138.1, 166.8, 167.5, 170.6, 173.0, 210.

Reductive Denitration of Paclitaxel-7, 2'-Dinitrate Ester

Paclitaxel-7, 2'-dinitrate 200 mg was dissolved in 3 ml of acetic acid and 500 mg of zinc powder was added. The mixture was stirred at room temperature for 30 minutes and then filtered through a celite bed to remove the zinc powder. Water was added to the acetic acid and the acid was neutralized with bicarbonate. The aqueous layer was extracted with dichloromethane twice and the organic layer was dried with sodium sulfate. After removal of the solvent 174 mg of paclitaxel was crystallized from diethyl ether and ligroin. All NMR spectra matched those of an authentic sample.

Reaction of Paclitaxel-7-2'-Dinitrate with NaBH_4

Paclitaxel-7-2'-dinitrate ester 200 mg was dissolved in 2 ml of methanol and excess NaBH_4 was added. This was stirred for 10 minutes at room temperature and the reaction was quenched with 1 N HCl. The methanol was partially removed and water and dichloromethane was added and partitioned. The organic layer was removed and the water layer was extracted twice more with dichloromethane. The combined water layers were washed with water once and then dried with sodium sulfate. TLC analysis showed two products, the faster was UV active but did not char with H_2SO_4 while the slower product gave a positive test in both cases. The evaporated residue was applied to a regular silica column and eluted with 5-15% acetone in benzene. Both compounds were isolated and

crystallized from diethyl ether and ligroin. The faster spot was determined to be the side chain alcohol nitrate ester (116) (34 mg) and the slower product was baccatin III-7-nitrate ester (117) (148 mg).

Side chain alcohol nitrate ester (116)

Colorless needles, ^1H NMR δ : 3.68 (t, 8.7Hz, 1H, O-H), 3.87 (d, 14.1Hz, 2H, 1-H) 5.20 (m, 1H, 2-H), 5.80 (dd, 3.0, 9.6Hz, 1H, 3-H), 6.77 (d, 9.0Hz, 1H, N-H), 7.36-7.60 (m, 8H, o,m,p-Ph, m,p-NBz), 7.81 (d, 6.9Hz, 2H, o-NBz). ^{13}C NMR δ : 52.0, 59.2, 83.7, 126.6, 127.2, 128.4, 128.6, 128.9, 129.3, 132.5, 136.7, 168.6.

Baccatin III-7-nitrate ester (117)

White crystalline powder, UV λ_{max} (CH₃OH): 231 nm, ^1H NMR δ : 1.10 (s, 3H, 17-H), 1.13 (s, 3H, 16-H), 1.80 (s, 3H, 19-H), 2.03 (m, 1H, 6-H β), 2.10 (s, 3H, 18-H), 2.20 (s, 3H, 10-OAc) 2.24 (m, 2H, 14-H α,β), 2.32 (s, 3H, 4-OAc), 2.71 (m, 1H, 6-H α), 4.07 (d, 6.9Hz, 1H, 3-H), 4.14 (d, 8.7Hz, 1H, 20-H β), 4.34 (d, 8.7Hz, 1H, 20-H α), 4.89 (t, 8.1Hz, 1H, 13-H), 5.00 (d, 8.7Hz, 1H, 5-H), 5.62 (d, 7.2Hz, 1H, 2-H), 5.80 (dd, 7.2, 10.5Hz, 1H, 7-H), 6.34 (s, 1H, 10-H), 7.49 (t, 7.8Hz, 2H, m-Bz), 7.62 (t, 7.5Hz, 1H, p-Bz), 8.10 (d, 7.2Hz, 2H, o-Bz). ^{13}C NMR δ : 10.9, 15.2, 20.2, 20.7, 22.5, 26.8, 32.6, 38.4, 42.7, 47.8, 55.4, 67.8, 74.1, 75.4, 76.2, 78.7, 80.2, 80.3, 83.6, 128.7, 129.1, 130.1, 131.7, 133.8, 145.3, 166.9, 169.5, 171.0, 200.9.

Selective Denitration of Paclitaxel-7, 2'-Dinitrate Ester

Paclitaxel-7, 2'-dinitrate 200 mg was dissolved in 2 ml of acetonitrile and 0.1 ml of 20% ammonium sulfide and stirred at room temperature for 2 minutes. Water was added and the mixture was extracted with diethyl ether twice. Removal of the ether left a white

solid (167 mg) which was determined to be paclitaxel-7-mononitrate ester (119). All NMR spectra matched those of an authentic sample.

Acetylation of 10-Deacetyl Paclitaxel-7- β -Xyloside-2'', 3'', 4'', 10, 2',-Pentanitrate Ester

10-Deacetyl paclitaxel-7- β -xyloside-2'', 3'', 4'', 10, 2'-tetranitrate 600 mg was dissolved in 25 ml of acetic anhydride and 120 mg of DMAP was added and the reaction was stirred at room temperature for overnight. Water was added as was NaHCO_3 with stirring until no further frothing was observed. The aqueous mixture was then extracted three times with dichloromethane and this organic layer was washed with a saturated NaCl solution, with water, and dried with Na_2SO_4 . The TLC analysis of the organic layer showed two major products which were slightly faster moving than the starting material. A couple of minor products were present but no starting material was seen. This material was ran on a regular silica column using 25% \rightarrow 40% ethyl acetate in ligroin. A total of 172 mg of the faster spot was isolated and crystallized from acetone and ligroin. This product was determined to be the di-enol acetate (122). A total of 146 mg of the slower spot was isolated and crystallized from dichloromethane. This product was determined to be the mono-enol acetate (121).

10-Deacetyl paclitaxel -7- β -xyloside-2'', 3'', 4'', 10-tetranitrate-di-enol acetate (122)

Clear colorless needles, mp 193-195°C, UV λ_{max} (CH_3OH): 229 nm, FABMS m/z : 1206 ($M + 1$), 703, 613, 522. ^1H NMR δ : 1.13 (s, 3H, 17-H), 1.19 (s, 3H, 16-H), 1.77 (s, 3H, 19-H), 2.03 (s, 3H, 18-H), 2.04 (m, 1H, 6-H β), 2.21 (s, 3H, OAc), 2.29 (m, 1H, 14-H β), 2.30 (s, 3H, OAc), 2.52 (m, 1H, 14-H α), 2.53 (s, 3H, OAc), 2.79 (m, 1H, 6-H α),

3.70 (dd, 5.1, 12.9Hz, 1H, 5''-H), 3.87 (d, 6.9Hz, 1H, 3-H), 4.18 (d, 8.4Hz, 1H, 20-H β), 4.23 (m, 1H, 7-H), 4.29 (d, 8.4Hz, 1H, 20-H α), 4.80 (d, 8.7Hz, 1H, 5-H), 4.81 (d, 3.8Hz, 1H, 1''-H), 4.97 (dd, 4.2, 5.7Hz, 1H, 2''-H), 5.07 (dd, 4.5, 8.7Hz, 1H, 4''-H), 5.26 (t, 5.7Hz, 1H, 3''-H), 5.65 (d, 7.2Hz, 1H, 2-H), 6.06 (t, 7.8Hz, 1H, 13-H), 6.44 (s, 1H, 10-H), 6.73 (d, 7.2Hz, 2H, o-NBz), 7.17 (t, 7.8Hz, 2H, m-NBz) 7.23-7.28 (m, 3H, o-Ph, p-NBz), 7.52-7.63 (m, 4H, m,p-Bz, p-Ph), 8.21 (d, 7.5Hz, 2H, o-Bz). ¹³C NMR δ : 10.7, 15.2, 20.3, 21.1, 21.7, 26.1, 26.5, 35.7, 36.0, 42.7, 46.0, 57.9, 59.3, 71.4, 72.7, 73.9, 74.1, 74.5, 76.4, 79.4, 80.0, 80.3, 82.2, 83.5, 98.6, 128.1, 128.3, 128.4, 128.5, 128.8, 129.2, 129.8, 130.4, 131.4, 132.6, 133.6, 133.7, 133.8, 135.0, 137.3, 142.7, 161.6, 167.0, 170.1, 172.0, 172.1, 174.6, 200.0.

10-Deacetyl paclitaxel -7- β -xyloside-2'', 3'', 4'', 10-tetranitrate-mono-enol acetate (121)

White crystalline powder, mp 178-180° C, UV λ_{\max} (CH₃OH): 232 nm, FABMS m/z: 1164 (M + 1), 326, 308. ¹H NMR δ : 1.15 (s, 3H, 17-H), 1.22 (s, 3H, 16-H), 1.77 (s, 3H, 19-H), 2.01 (s, 3H, 18-H), 2.04 (m, 1H, 6-H β), 2.10 (s, 3H, OAc), 2.11 (m, 1H, 14-H β), 2.39 (s, 3H, OAc), 2.56 (m, 1H, 14-H α), 2.83 (m, 1H, 6-H α), 3.72 (dd, 5.1, 12.9Hz, 1H, 5''-Heq), 3.81 (d, 7.2Hz, 1H, 3-H), 4.16 (d, 8.7Hz, 1H, 20-H β), 4.16 (m, 1H, 7-H), 4.23 (dd, 3.6, 15.6Hz, 1H, 5''-Hax), 4.33 (d, 8.7Hz, 1H, 20-H α), 4.83 (d, 3.6Hz, 1H, 1''-H), 4.87 (d, 8.7Hz, 1H, 5-H), 4.97 (dd, 3.9, 6.0Hz, 1H, 2''-H), 5.07 (dd, 5.1, 9.3Hz, 1H, 4''-H), 5.26 (t, 5.7Hz, 1H, 3''-H), 5.69 (d, 6.9Hz, 1H, 2-H), 6.10 (t, 8.1Hz, 1H, 13-H), 6.44 (s, 1H, 10-H), 7.39-7.52 (m, 9H, m-Bz, o,m,p-Bz, m-NBz), 7.57 (m, 1H, p-NBz), 7.61 (m, 1H, p-Bz), 7.94 (d, 7.2Hz, 2H, o-NBz), 8.06 (d, 7.2Hz, 2H, o-

Bz), 11.41 (s, 1H, N-H). ^{13}C NMR δ : 10.5, 15.3, 20.3, 21.1, 21.9, 26.2, 35.8, 36.2, 42.8, 45.8, 57.9, 59.1, 71.5, 72.3, 73.7, 73.8, 74.1, 76.4, 79.0, 80.3, 80.4, 82.2, 83.3, 98.6, 120.9, 127.6, 127.7, 128.2, 128.7, 128.8, 128.9, 129.6, 130.0, 130.9, 131.7, 132.8, 133.0, 133.9, 143.2, 147.7, 164.6, 165.0, 166.9, 169.6, 170.7, 200.0.

Reaction of Paclitaxel-7, 2'-Dinitrate Ester with NaN_3

Paclitaxel-7, 2'-dinitrate ester 200 mg was dissolved in 4 ml of acetonitrile and 200 mg of sodium azide was added. This mixture was stirred at room temperature overnight. At that point the acetonitrile was partially evaporated and the residue was partitioned between water and dichloromethane. The organic layer was removed and the water layer was partitioned twice more with dichloromethane. The combined organic layers were washed once with water and then dried with sodium sulfate. TLC analysis showed no starting material and two products. The faster moving product was UV active but did not char with 1 N H_2SO_4 while the slower product did give a positive result in both cases. The solvent was removed and the residue was put on a regular silica column with 5-15% acetone in benzene. Both products were cleanly isolated and the slower product crystallized from diethyl ether and ligroin while the faster compound crystallized upon evaporation of the fraction solvents. The faster spot was determined to be dibenzamide (**125**) (20 mg) and the slower product was baccatin III-7-nitrate ester (**126**) (152 mg).

Dibenzamide (125)

Colorless needles, mp 150-151° C, UV λ_{max} (CH_3OH): 242 nm, IR (KBr): 3240, 1770, 1475, 1225, 1115, 705 cm^{-1} , ^1H NMR δ : 7.51 (t, 7.5Hz, 4H, m-NBz), 7.61 (t,

7.2Hz, 2H, p-NBz), 7.87 (d, 7.8Hz, 4H, o-NBz), 8.99 (br s, 1H, N-H). ^{13}C NMR δ : 127.9, 128.9, 133.1, 133.3, 166.4.

Baccatin III-7-nitrate ester (126)

see compound 117

Synthesis of 2'-Oxo Paclitaxel-7-Nitrate Ester from Paclitaxel-7, 2'-Dinitrate Ester

Paclitaxel-7, 2'-dinitrate ester 200 mg was dissolved in 3 ml of DMF and 200 mg of NaN_3 was added. Immediately the solution turned a deep pink color while stirring at room temperature. After 10 minutes water was added and the color dissipated and a white solid precipitated from the solution. This solid was filtered and dried to yield 182 mg of >95% keto-ester (**124**). White amorphous powder, UV λ_{max} (CH_3OH): 230 nm, IR (KBr): 2960, 1725, 1640, 1270, 1225, 1060, 1020, 830, 700 cm^{-1} . FABMS: 897 (82%, $\text{M}+1$), 614 (13%), 554 (35%), 307 (16%), 284 (55%), 210 (100%). For ^1H NMR see below compound.

Synthesis of 2'-Oxo Paclitaxel-7-Nitrate Ester from Paclitaxel-7-Mononitrate Ester

Paclitaxel-7-mononitrate ester 200 mg was dissolved in 3 ml of acetone and a few drops of 3 N Jones reagent was added. This solution was stirred at 60° C and checked by TLC every 30-60 minutes and more Jones reagent was added as needed. After about 6 hours the TLC showed about a 40% conversion and the reaction didn't seem to proceed any further so the acetone was evaporated and the residue was taken up in water. The water layer was extracted with dichloromethane twice and the combined organic layers were washed with water once. The dried organic layer was evaporated to dryness and the residue was put on a silica column and eluted with 5-15% acetone in benzene. A total of

63 mg of product (**128**) was obtained. White amorphous powder, ^1H NMR δ : 1.21 (s, 3H, 17-H), 1.26 (s, 3H, 16-H), 1.79 (s, 3H, 19-H), 1.99 (s, 3H, 18-H), 2.05 (m, 1H, 6-H β), 2.12 (m, 1H, 14-H β), 2.18 (s, 3H, 4-OAc), 2.20 (s, 3H, 10-OAc), 2.34 (m, 1H, 14-H α), 2.70 (m, 1H, 6-H α), 4.01 (d, 6.6Hz, 1H, 3-H), 4.11 (d, 8.4Hz, 1H, 20-H β), 4.31 (d, 8.7Hz, 1H, 20-H α), 4.97 (d, 8.7Hz, 1H, 5-H), 5.64 (d, 6.9Hz, 1H, 2-H), 5.79 (dd, 7.5, 10.8Hz, 1H, 7-H), 6.20 (t, 8.1Hz, 1H, 13-H), 6.31 (s, 1H, 10-H), 6.42 (d, 5.4Hz, 1H, 3'-H), 7.14 (d, 5.4Hz, 1H, N-H), 7.43-7.51 (m, 10H, m-Bz, o,m,p-Ph, m,p-NBz), 7.62 (t, 6.3Hz, 1H, p-Bz), 7.84 (d, 7.2Hz, 2H, o-NBz), 8.03 (d, 7.2Hz, 2H, o-Bz).

Acetylation of 2'-Oxo-Paclitaxel-7-Mononitrate Ester

A total of 300 mg of 2'-oxo-paclitaxel-7-mononitrate ester was dissolved in 2 ml of acetic anhydride and 50 mg of DMAP was added and the solution was stirred at room temperature for 18 hours. Water was then added to the solution and sodium bicarbonate was added slowly with stirring. After the release of CO_2 stopped the solution was extracted twice with dichloromethane. The combined organic layers were washed with 0.1 N NaOH, 0.1 N HCl, and water successively, dried with sodium sulfate and evaporated to a solid residue. This residue was put on a silica column and eluted with 0-10% acetone in dichloromethane. Two major products were eluted, the faster being the C-2'-C-3' monoenol acetate (**130**) which was crystallized from diethyl ether and ligroin to yield 175 mg, and the slower product was determined to probably be one of two possible enols (**131**, **132**). This was also crystallized from diethyl ether and ligroin to yield 38 mg.

Paclitaxel-7-mononitrate ester-2'-3'-enol acetate (130)

White crystalline powder, mp 175-178° C, UV λ_{max} (CH₃OH): 233 nm, FABMS m/z: 939 (39%, M+1), 614 (31%), 554 (59%), 326 (27%), 308 (100%), 266 (79%), 237 (35%), 204 (83%). ¹H NMR δ : 1.18 (s, 3H, 17-H), 1.26 (s, 3H, 16-H), 1.82 (s, 3H, 19-H), 1.97 (s, 3H, 18-H), 2.05 (m, 3H, 6-H β), 2.10 (m, 1H, 14-H β), 2.11 (s, 3H, 2'-OAc), 2.21 (s, 3H, 10-OAc), 2.41 (s, 3H, 4-OAc), 2.59 (m, 1H, 14-H α), 2.70 (m, 1H, 6-H α), 4.02 (d, 6.6Hz, 1H, 2-H), 4.15 (d, 8.7Hz, 1H, 20-H β), 4.34 (d, 8.7Hz, 1H, 20-H α), 4.98 (d, 9.3Hz, 1H, 5-H), 5.67 (d, 6.9Hz, 1H, 2-H), 5.75 (dd, 7.2, 10.5Hz, 1H, 7-H), 6.09 (t, 8.4Hz, 1H, 13-H), 6.33 (s, 1H, 10-H), 7.37-7.63 (m, 11H, m,p-Bz, o,m,p-Ph, m,p-NBz), 7.94 (d, 7.2Hz, 2H, o-NBz), 8.06 (d, 6.9Hz, 2H, o-Bz), 11.43 (br s, 1H, N-H). ¹³C NMR δ : 10.9, 14.8, 20.3, 20.6, 20.8, 21.8, 26.5, 32.6, 36.3, 43.1, 47.4, 55.4, 71.8, 74.0, 74.7, 76.2, 79.1, 80.0, 80.4, 83.5, 121.1, 127.6, 127.7, 128.2, 128.7, 128.9, 129.0, 129.5, 130.0, 131.7, 132.8, 132.9, 133.1, 133.9, 141.0, 147.4, 164.8, 165.0, 166.8, 169.4, 169.7, 170.4, 200.5.

Paclitaxel-7-nitrate ester enol (131, 132)

White crystalline powder, mp 176-179° C, FABMS m/z: 939 (23%, M+1), 614 (18%), 554 (29%), 460 (25%), 410 (52%), 308 (29%), 266 (38%), 136 (27%), 105 (100%). ¹H NMR δ : 1.09 (s, 3H, 17-H), 1.15 (s, 3H, 16-H), 1.28 (s, 3H, 19-H), 1.77 (s, 3H, 18-H), 2.04 (m, 1H, 6-H β), 2.17 (s, 3H, OAc), 2.23 (m, 1H, 14-H β) 2.28 (s, 3H, OAc), 2.40 (m, 1H, 14-H α), 2.41 (s, 3H, OAc), 2.63 (m, 1H, 6-H α), 3.92 (d, 7.2Hz, 1H, 3-H), 4.15 (d, 8.4Hz, 1H, 20-H β), 4.28 (d, 8.4Hz, 1H, 20-H α), 4.92 (d, 8.4Hz, 1H, 5-H), 5.59 (d, 7.2Hz, 1H, 2-H), 5.71 (dd, 7.2, 10.8Hz, 1H, 7-H), 5.91 (t, 9.6Hz, 1H, 13-H),

6.19 (s, 1H, 10-H), 7.47-7.63 (m, 11H, m,p-Bz, o,m,p-Ph, m,p-NBz), 7.84 (d, 7.2Hz, 2H, o-NBz), 8.12 (d, 7.2Hz, 2H, o-Bz). ^{13}C NMR δ : 11.1, 13.3, 20.6, 20.7, 21.3, 22.7, 26.1, 32.4, 35.8, 42.9, 47.2, 55.1, 71.9, 74.3, 74.6, 76.2, 79.4, 79.8, 79.9, 83.7, 127.5, 128.6, 128.7, 129.0, 129.1, 129.3, 129.4, 130.3, 130.7, 132.2, 132.7, 132.8, 133.5, 133.7, 133.8, 141.4, 163.4, 163.7, 166.7, 168.5, 169.4, 171.5, 200.6.

CHAPTER 4 SYNTHESIS OF ANALOGUES WITH POTENTIALLY IMPROVED WATER SOLUBILITY

Introduction

In spite of paclitaxel's great promise in treatment of refractory and untreatable human neoplasms it is afflicted with formulation and systemic administration problems. These problems stem from its extreme low solubility in water which has been reported as low as 0.25 $\mu\text{g/ml}$ (Ali et al., 1995). Consequently, special formulations requiring excipients such as Cremophore EL® have been necessary for intravenous administration. In the case of paclitaxel the amount of Cremophore EL® required to administer the therapeutic dose (135-200 mg/m^2) represents the highest amount ever to be used with any drug. Exposure to the large amounts of Cremophore EL® has produced major hypersensitivity reactions in patients. It is perceived that such adverse effects are vehicle related, since it is well documented that Cremophore EL® alone causes hypotension and histamine release in dogs. The high incidences and severity of these side effects to paclitaxel almost led to the termination of some earlier Phase I clinical trials. However, prolonged infusions and prophylactic medications with antihistamines and corticosteroids have avoided the adverse episodes and allowed continuation of clinical use of Cremophore EL® formulation. Still, because of these problems and the additional medications needed, an equally potent but more water-soluble analogue of paclitaxel is desirable. This area has

been the focus of much research and various methods have been developed. A variety of water-soluble analogues have been developed which contain esterase or phosphatase-cleavable pro-moieties. However, these prodrugs are liable to exhibit unstable efficacy because of variation in the enzymatic activity among patients. Therefore it would be very advantageous to develop non-prodrugs of paclitaxel with satisfactory stability *in vivo*, high water-solubility, and potent antitumor activity. This laboratory has studied two possible approaches to this type of analogue and they are discussed below.

Synthesis of Analogues Starting from 10-Deacetyl Paclitaxel-7-Xyloside

It was discussed in Chapter 2 that 10-deacetyl paclitaxel-7-xyloside is actually the most predominate taxane found in the bark of *Taxus brevifolia* occurring in a yield of 0.1% which is 2.5 times as much as paclitaxel. Other xylosides (10-deacetyl paclitaxel-C-7-xyloside and 10-deacetyl cephalomannine-7-xyloside) are also found in high yields and can be converted into 10-deacetyl paclitaxel-7-xyloside by modification of the amide function. Although these xylosides have not been reported to a great extent in other *Taxus* species, this does not mean they are not present since most published isolation procedures are not ideal for obtaining these xylosides. It has also been reported that these xylosides inhibit the *in vitro* disassembly of microtubules from mammalian brain at lower concentrations than paclitaxel (Lataste et al., 1984). This is shown in Table 4-1. In light of this fact and the assumption that these xylosides would undoubtedly have greater water-solubility than paclitaxel, it is interesting that no reports have seriously examined the possibility of using the xylosides as alternatives to paclitaxel clinically. One reason for this

may be the fact that although the xylosides are more active *in vitro*, they have been reported to be less active in cell culture toxicity assays (Rao, 1993). Obviously the xylose unit is either causing a decrease in uptake into the cell or to the site of action or the xylose unit is serving as a site for metabolism which serves to inactivate the compound.

Table 4-1: ID₅₀ Values of Paclitaxel and Xylosides in Tubuline Assay

Compound	ID ₅₀ for disassembly of microtubules, μ M
Paclitaxel	0.5
Paclitaxel-7-Xyloside	0.2
10-Deacetyl Paclitaxel-7-Xyloside	0.3
Cephalomannine-7-Xyloside	0.25

With this information in hand, the goal was then to devise a scheme in which the xylose unit would be changed in such a way as to hopefully increase the cell culture cytotoxicity over the parent xyloside as well as retain an assumed water-solubility advantage over paclitaxel. As discussed in Chapter 3, the xylose unit in these xylosides can be easily oxidized with periodate to the dialdehyde. It should be mentioned that this reaction occurs without interfering with any other part of the molecule including the α -hydroxy ketone function at C-9 and C-10 of 10-deacetyl paclitaxel-7-xyloside since this general function type is usually cleaved with periodate. The dialdehyde that is formed upon oxidation and loss of one carbon actually exist as an equilibrium between 3 different structures (143, 144, 145) but since it is the dialdehyde (144) which serves as the electrophile the oxidation product will be referred to as the dialdehyde (Figure 4-1).

Once this dialdehyde is obtained by stirring the xyloside in an aqueous acidic solution also containing NaIO₄, it can then be condensed with a variety of nucleophiles. Among these are carbon nucleophiles such as β -dicarbonyl compounds as well as

nitroalkanes. The dialdehyde could also undergo reductive amination in the presence of an amine and a reducing agent. The latter method would result in the formation of morpholino analogues and is well documented, while the former reactions would result in the formation of a tetrahydropyran ring. In each case an ionizable group could be incorporated into the function by using these methods assuming reduction of the nitro group to an amine following the condensation (Figure 4-1). Indeed each of these methods were exploited in this work however, because of the unavailability of a large number of β -dicarbonyls and nitroalkanes and the apply supply of amines, the reductive amination procedure received the most attention. It should also be mentioned that only 10-deacetyl paclitaxel-7-xyloside was used in this work because on the large supply of this compound on hand.

Concerning condensation with β -dicarbonyls, only one reaction was attempted and this was condensation of the dialdehyde (**150**) with malonic acid (**151**) (Figure 4-2). This reaction proceeded smoothly by refluxing the dialdehyde with 1.5 eq. of malonic acid in pyridine/piperidine. However, the expected diacid was not the product isolated. Instead, based on NMR and mass spectroscopy it was concluded that decarboxylation and loss of H_2O had taken place thus resulting in the α , β -unsaturated acid (**152**) as the product (Figure 4-2). The stereochemistry at C-2'' was not determined.

Condensation with a nitroalkane was also only attempted with one such reagent and this was nitromethane (**154**) (Figure 4-3). This reaction proceeded by using a 1 : 1 mixture of CH_2Cl_2 and Et_3N as solvent/base and dissolving the dialdehyde (**153**) along

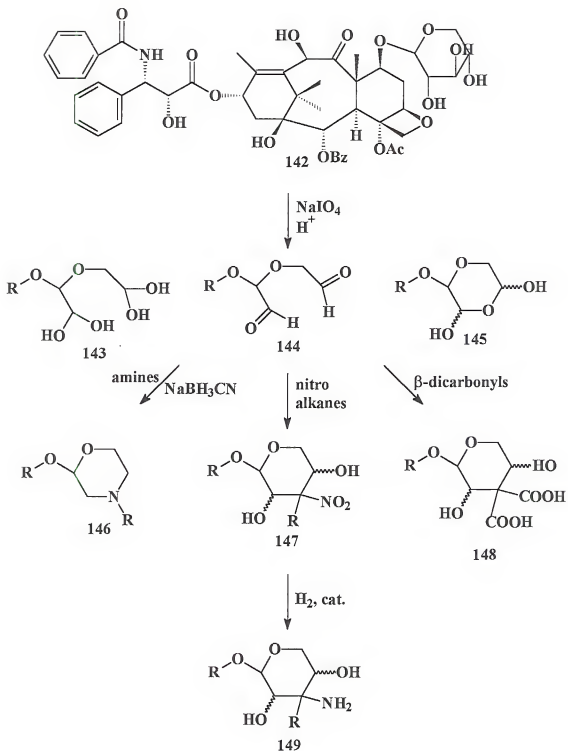


Figure 4-1: Synthesis of Ionizable Analogues

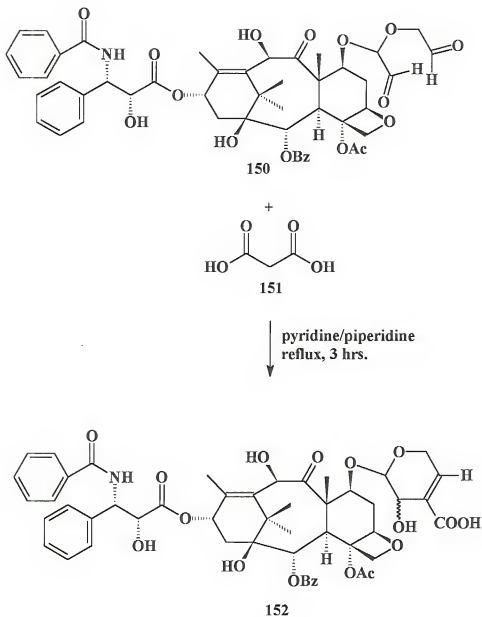


Figure 4-2: Condensation with Malonic Acid

with 2.5 eq. of nitromethane and stirring at room temperature for overnight. Although essentially all the starting material disappeared the reaction was not a clean one being that at least three products were formed. This result was somewhat expected since the reaction

generates three stereocenters thus a variety of isomers are possible. The major product was isolated and based on the mass spectrum and ^{13}C NMR it was concluded that this was the desired product with unknown stereochemistry (**155**). Overlapping peaks in the ^1H NMR spectrum made it impossible to determine cis/trans configuration based on coupling constants. It is not unreasonable to assume that the isolated major product is the all-trans equatorial conformer since that would be the thermodynamically favored product.

Aside from having a greater number of nucleophiles to choose from, the reductive amination procedure also offers the advantage of not generating asymmetric carbons. Indeed this reaction actually converts two asymmetric carbons into methylene carbons. The reducing agent chosen for these reactions was sodium cyanoborohydride. This reagent has the advantage over sodium borohydride since it can be used in mildly acidic conditions therefore no loss of the side chain occurs as with sodium borohydride. Also this reagent has been shown to perform ideally in reductive amination reactions.

Initially the goal was to use a variety of amines, both aromatic and aliphatic, as well as amino acids in these reactions. Attachment of di- and tripeptides was envisioned as well. Unfortunately aliphatic amines including common amino acids did not condense with the dialdehyde under the conditions used. Instead, the dialdehyde (**156**) function was reduced to the corresponding diol (**157**) (Figure 4-4), and this occurrence was probably due to the increased steric hindrance at the sp^3 α -carbon as opposed to the sp^2 α -carbon of an aromatic amine. Although this problem may have been overcome by varying the conditions, time did not allow for this investigation.

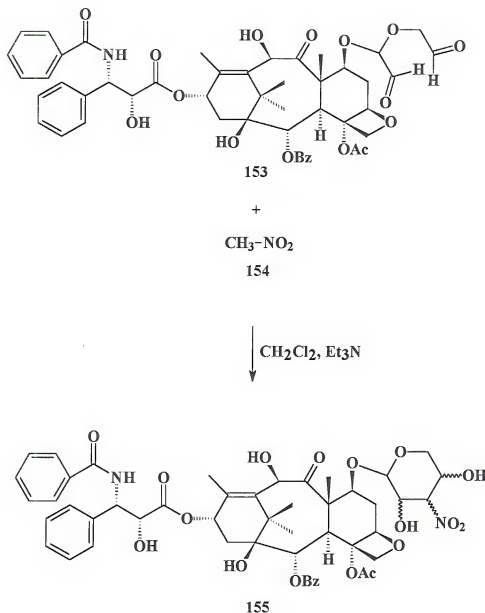


Figure 4-3: Condensation with Nitromethane

When using aromatic amines however this problem was not encountered and a smooth reaction occurred. The dialdehyde (158) was added with 5 eq. of amine in 2 : 1 CH_3OH and acetic acid and an excess of NaCNBH_3 was added and stirred for 1.5 hours.

These conditions gave clean products which were easily purified by column chromatography. The following amines were used: p-aminobenzoic acid, p-aminosalicylic acid, m-aminosalicylic acid, p-nitroaniline, and p-aminobenzenesulfonamide which was obtained by reducing p-nitrobenzenesulfonamide with Sn and HCl. These amines generated the corresponding morpholino analogues (160-164) (Figure 4-5).

Attempted Synthesis of Taxane Glycosides

An alternative method of preparing analogues with increased water-solubility was also studied in this work. This method involved attaching sugar units to the taxane moiety and thereby forming glycosides. This would certainly increase the water-solubility and quite possibly have a positive effect on the activity of the drug since it is well known that carbohydrates play a vital role in molecular recognition. The only taxane glycoside that had been studied was the naturally occurring xylosides and although they displayed very good activity in tubuline assays but lost activity in cell culture as mentioned, it was hoped that attaching different sugars would overcome this problem. Also the possibility existed that if a di- or trisaccharide could be attached to paclitaxel or one of its close analogues then possibly it would become orally available. This type of behavior is seen with the cardiac glycosides in which if the sugars are removed the compound is no longer orally active. As of this writing two papers have been published in which a taxane has been linked with a carbohydrate unit to increase the water-solubility (Paradis & Page, 1998; Takashi et al., 1998).

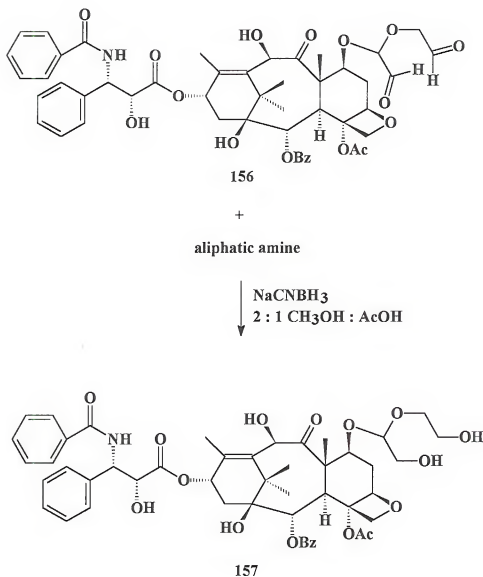
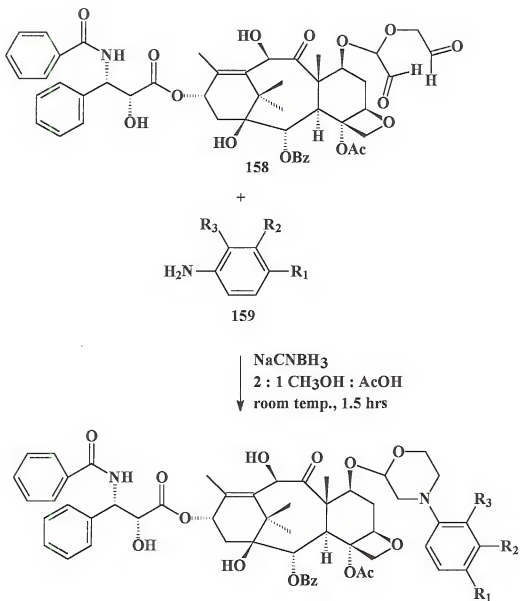


Figure 4-4: Dialdehyde Reduction to Diol

Three methods of glycosylation were to be studied in this work are these are; 1) the Koenigs-Knorr method, 2) the trichloroacetimidate method, and 3) the sulphoxide method. These will now be briefly discussed.

The Koenigs-Knorr method was first introduced in 1901 and has since been a mainstay in glycosylation chemistry (Toshima & Tatsuta, 1993). In its classical form it



- 160 $R_1 = \text{COOH}$, $R_2 = \text{H}$, $R_3 = \text{H}$
 161 $R_1 = \text{COOH}$, $R_2 = \text{OH}$, $R_3 = \text{H}$
 162 $R_1 = \text{H}$, $R_2 = \text{COOH}$, $R_3 = \text{OH}$
 163 $R_1 = \text{NO}_2$, $R_2 = \text{H}$, $R_3 = \text{H}$
 164 $R_1 = \text{SO}_2\text{NH}_2$, $R_2 = \text{H}$, $R_3 = \text{H}$

Figure 4-5: Reductive Aminations

involves using a suitable protected glycosyl bromide or chloride as the glycosyl donor. The reaction is facilitated by using various heavy metal salts as activating agents. These metals technically do not function as catalyst since an equivalent amount is needed. Some of the more common heavy metal salts include; AgOTf, Ag₂O, AgClO₄, AgNO₃, Hg(CN)₂, and HgBr₂. The silver salts are the strongest activators with AgOTf being the strongest. In some cases an acid scavenger may also be used and examples of these include HgO, CdCO₃, and *s*-collidine.

The trichloroacetimidate-mediated glycosylation was announced in 1980 as an alternative method to the classical Koenigs-Knorr procedure and now appears to be one of the most ideal glycosylation protocol (Toshima & Tatsuta, 1993). This method involves using a suitably protected anomeric trichloroacetimidate as the glycosyl donor. This donor is prepared by condensing a protected 1-hydroxy sugar with trichloroacetonitrile in the presence of base. Depending on which base is used, one can prepare the α or β epimer using kinetic or thermodynamic control. This is not possible with halides. This reaction is smoothly promoted by the catalytic use of BF₃-Et₂O, TMSOTf, CCl₃CHO, PTSA, and ZnBr₂.

The sulphoxide method is the newest of the three methods being first reported in the literature in 1989 (Kahne et al., 1989). This method involves the use of a suitably protected glycosyl sulphoxide as the glycosyl donor (usually a phenyl sulphoxide), an equimolar amount of triflic anhydride as the activator, and 2, 6-di-*tert*-butyl-4-methylpyridine as an acid scavenger. The stereochemistry of the resulting glycoside can be controlled by varying the reaction solvent. This method has been shown to be very

applicable to unreactive and hindered nucleophiles and has been the basis for developing a combinatorial process involving oligosaccharides.

Acetyl protected glycosyl donors for each of these methods were prepared using D-glucose as the sugar as shown in Figure 4-6. Initially D-glucose (165) is acetylated with acetic anhydride and pyridine to give the pentaacetate (166) in near quantitative yields. Next, bromine was introduced at the anomeric carbon by treating the pentaacetate with 30% HBr in acetic acid which gave yields around 80%. This product (167) was used as the Koenigs-Knorr donor and also as a starting material for the other two donors. To synthesize the trichloroacetimidate donor the bromide was hydrolyzed in aqueous acetonitrile with $\text{Hg}(\text{CN})_2$ added. This gave a quantitative yield. The α -trichloroacetimidate (169) was produced from this by adding trichloroacetonitrile and using K_2CO_3 as base in CH_2Cl_2 in a yield of about 75%. The sulphoxide donor was prepared by first by making the phenylthioglycoside (170) using the bromide, thiophenol, and KOH as base. This reaction gave near quantitative yields. The sulfide was then oxidized to the sulphoxide (171) with mCPBA to give near quantitative yields (Figure 4-6).

Once these donors were in hand a series of test glycosylations were performed using β -sitosterol (172) and benzyl alcohol (173) as aglycones to determine the best conditions. Unfortunately the sulphoxide method was never attempted due to a lack of the triflic anhydride activator. However both the Koenigs-Knorr and trichloroacetimidate methods were attempted and met with success. In terms of the Koenigs-Knorr method, β -glycosides were formed with both of these aglycones in moderate yields with the benzyl

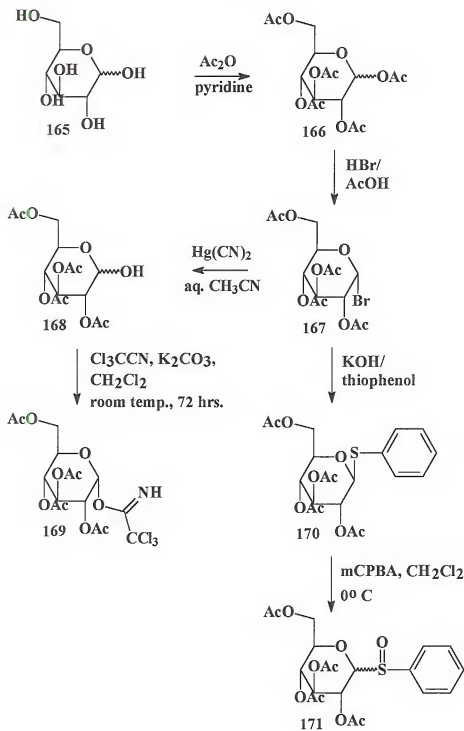


Figure 4-6: Synthesis of Glycosyl Donors

alcohol reaction giving the best results. These reactions were conducted at room temperature in CH_2Cl_2 (Figure 4-7). The β orientation was determined based on the anomeric carbon and proton chemical shifts as well as comparison to the acetylated naturally occurring β -sitosterol- β -glucoside. Of the activators that were available, $\text{Hg}(\text{CN})_2$, AgNO_3 , and ZnCl_2 were the best. None of the stronger silver Lewis acids were available. The trichloroacetimidate method was also attempted with both of these substrates using PTSA and $\text{BF}_3\cdot\text{Et}_2\text{O}$ as the activators and CH_2Cl_2 as solvent. The reactions were performed at room temperature (Figure 4-8). Although a substantial amount of hydrolysis of the donor did occur, it was obvious that this method was superior giving greater yields in a shorter amount of time. The products formed also contained the β orientation.

Since these results were encouraging, similar reactions were attempted with paclitaxel. Unfortunately none of these reactions were successful. As mentioned earlier paclitaxel and related taxanes are quite unstable to acids, both mineral and Lewis. Under these conditions a variety of rearrangements have been documented. When the weaker Lewis acids such as $\text{Hg}(\text{CN})_2$ were used in conjunction with the Koenigs-Knorr method no reaction took place at all. This was probably due to the steric hindrance involved at the C-2' and C-7 hydroxyls of paclitaxel. Therefore faced with this problem the use of stronger Lewis acids would be required; however, when this was attempted with AgNO_3 and ZnCl_2 rearrangements did occur. In most cases the rearranged product was not identified but in the case of AgNO_3 the product was identified as the rearranged taxane **183** when paclitaxel-2'-acetate (**182**) was used as the aglycone (Figure 4-9). Therefore, in

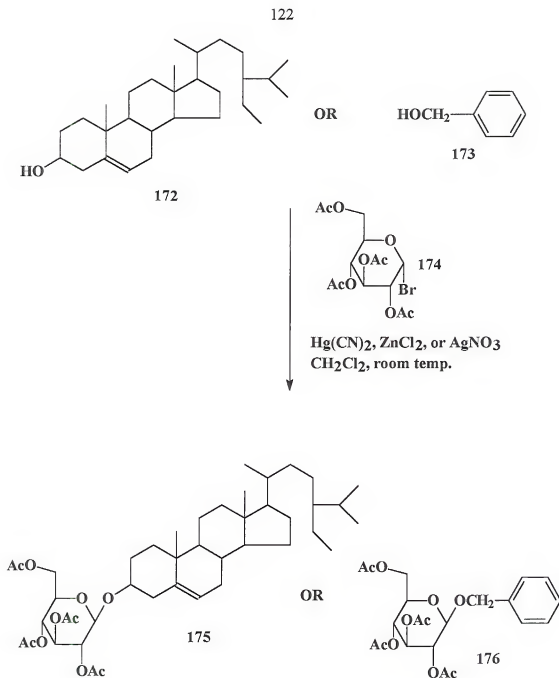


Figure 4-7: Koenigs-Knorr Glycosylation

order to take advantage of less hindered hydroxyls, 10-deacetyl paclitaxel-7-xyloside was used as the substrate hoping the reaction would occur on one of the xylose hydroxyls. However as before no reaction took place with the weaker Lewis acids. In an attempt to

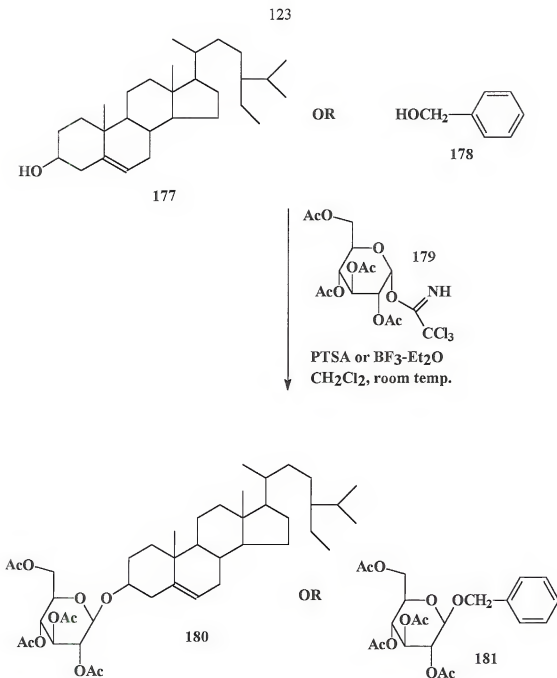
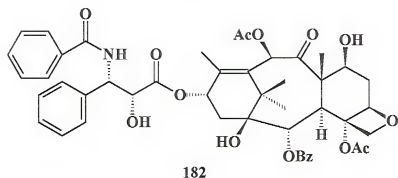


Figure 4-8: Trichloroacetimidate Glycosylation

produce a primary hydroxyl which should be easily glycosylated, 10-deacetyl paclitaxel-7-xyloside was oxidized to the dialdehyde as before and this dialdehyde was subsequently reduced with NaCNBH₃ to the diol (Figure 4-4). Unfortunately when this substrate was



acetobromoglucose
 AgNO_3
 CH_2Cl_2 , room temp.

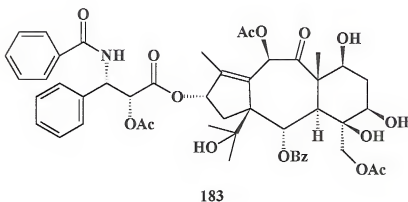


Figure 4-9: Rearrangement of 2'-Acetyl Paclitaxel

subjected to the Koenigs-Knorr reaction the main product was loss of the diol function due to hydrolysis to give 10-deacetyl paclitaxel.

A few reactions of the trichloroacetimidate type were also investigated with paclitaxel and various analogues but no favorable reactions occurred. Unfortunately due to time constraints and other factors (death of professor) this area of research was ceased. The author does believe that if more controlled conditions were used (dry solvents, nitrogen atmosphere, low temperatures, less amounts of Lewis acids, etc.) these reactions could be successful. Also the sulphoxide method may be better than the two that were investigated and deserves attention that the author could not give it.

L1210 Cytotoxicity of Analogues

The L1210 assay is commonly used to test compounds for their cytotoxicity and is a screen for compounds with anticancer activity. These murine leukemia cells have a very rapid doubling time of about 12 hours. Thus, they provide a convenient and relatively reliable means for the determination of the cytotoxicity of many compounds (Thayer et al., 1971).

The following compounds were chosen to be tested on the L1210 assay: paclitaxel, 10-deacetyl paclitaxel-7-xyloside, malonic acid condensation product **152**, nitromethane condensation product **155**, reductive amination products **160-164**, and the reduced diol **157**. The ionizable compounds were tested in their neutral form. Table 4-2 shows the results of the assay.

As Table 4-2 shows, there was a wide range of activity in this series of compounds. Although none of the compounds tested is nearly as active as paclitaxel, 4 of these showed greater activity than 10-deacetyl paclitaxel-7-xyloside. Of the reductive

amination products (**160-164**) the only compounds which is less active than the xyloside is the m-aminosalicylic acid product (**162**) which has a substituent ortho to the amino group. The p-aminosalicylic acid product (**161**) however is much more active, thus indicating that ortho substituents are not well tolerated. However more work needs to be performed to verify this.

Table 4-2: L1210 Cytotoxicity of Paclitaxel and Analogues

Compd.	IC ₅₀ (ppm)	IC ₅₀ (μM)	Compd./Paclitaxel
Paclitaxel	0.0089	0.01	****
10-Deacetyl Paclitaxel-7-Xyloside	0.37	0.39	39
152	3.31	3.47	347
155	0.89	0.92	92
160	0.048	0.047	4.7
161	0.095	0.092	9.2
162	2.00	1.94	194
163	0.071	0.071	7.1
164	0.13	0.12	12
157	0.93	1.02	102

Experimental

All reactions were monitored by silica gel 60 HF₂₅₄ TLC to ensure completion of the reaction. All NMR spectra were recorded using either a Varian VXR-300 or a Varian Gemini-300 spectrophotometer using CDCl₃ as solvent. Infrared spectra were obtained using a Perkin-Elmer 1420 ratio recording spectrophotometer. Ultraviolet spectra were obtained using a Shimadzu UV160U recording spectrophotometer. Mass spectra were recorded on a Finnigan Mat 950 Q spectrometer. Melting points were obtained by using a Fisher melting point apparatus. Column chromatography was used in conjunction with 100-200 mesh silica gel.

Oxidation of Xyloside with Periodate

10-Deacetyl paclitaxel -7- β -xyloside (1.0 g) was dissolved in 10 ml of 1 : 1 THF and water and 2 ml of 1 N H₂SO₄ was added. This was followed by 0.71 g of NaIO₄ and the mixture was stirred overnight at room temperature. The mixture was diluted with water and the solid which precipitated (920 mg) was filtered and dried.

Condensation of Dialdehyde with Malonic Acid

A total of 200 mg of dialdehyde was dissolved in 3 ml of pyridine and 5 drops of piperidine was also added. To this mixture was added 20 mg of malonic acid and the mixture was refluxed for 3 hours. At this point the mixture was diluted with CH₂Cl₂ and washed with 0.1 N HCl 3 times and with water twice. The organic layer was then dried with Na₂SO₄ and evaporated to a residue. The residue was separated on a silica column using 30-50% ethyl acetate in ligroin with a few drops of acetic acid as the mobile phase. A total of 123 mg of product was crystallized from diethyl ether, ligroin, and acetone. Yellowish white crystalline powder, mp 172-174° C (dec.), UV λ_{\max} (CH₃OH): 225 nm, FABMS m/z: 954 (M + 1), 795, 669, 651, 105. ¹H NMR δ (some DMSO): 1.09 (s, 3H, 17-H), 1.18 (s, 3H, 16-H), 1.75 (s, 3H, 19-H), 1.85 (s, 3H, 18-H), 1.87 (m, 1H, 14-H β), 2.04 (m, 1H, 6-H β), 2.30 (m, 1H, 4-H α), 2.39 (s, 3H, 4-OAc), 2.78 (m, 1H, 6-H α), 3.89 (d, 6.3Hz, 1H, 3-H), 4.05 (s, 1H, 2''-H), 4.14-4.30 (m, 5H, 7-, 20 α -, 20 β -, 5''ax-, 5''eq-H), 4.73 (s, 1H, 1''-H), 4.83 (d, 3.0Hz, 1H, 2'-H), 4.94 (d, 8.7Hz, 1H, 5-H), 5.25 (s, 1H, 10-H), 5.65 (d, 6.9Hz, 1H, 2-H), 5.80 (dd, 2.1, 8.7Hz, 1H, 3'-H), 6.21 (t, 8.1Hz, 1H, 13-H), 7.04 (s, 1H, 4''-H), 7.31-7.52 (m, 10H, m-Bz, m,p-NBz, o,m,p-Ph), 7.59 (t, 7.5Hz, 1H, p-Bz), 7.60 (d, 9.3Hz, 1H, N-H), 7.77 (d, 7.2Hz, 2H, o-NBz), 8.10 (d, 7.5Hz, 2H, o-

Bz). ^{13}C NMR δ (some DMSO): 10.6, 14.1, 20.6, 22.5, 26.5, 35.1, 35.6, 43.0, 46.5, 55.2, 56.9, 60.7, 63.2, 72.0, 73.3, 74.4, 74.7, 76.5, 78.4, 80.8, 80.9, 84.0, 101.9, 127.0, 127.1, 127.7, 128.0, 128.2, 128.2, 128.5, 128.6, 128.7, 129.3, 130.1, 131.8, 133.5, 133.6, 135.9, 138.1, 138.3, 139.6, 166.6, 167.8, 168.3, 170.4, 172.7, 209.4.

Condensation of Dialdehyde with Nitromethane

A total of 200 mg of dialdehyde and 35 mg of nitromethane was dissolved in a 1 : 1 mixture of benzene and triethylamine and this mixture was stirred for 4 days at room temperature. At this point the reaction mixture was evaporated to a residue under reduced pressure and this residue was run on a silica column using 10-30% acetone in benzene as the mobile phase. A total of 45 mg of amorphous product was obtained as the major product. White amorphous powder, UV λ_{max} (CH_3OH): 228 nm, FABMS m/z : 974 ($\text{M} + 2$), 956, 913, 689, 670, 603, 286, 154, 136, 105, 81. ^{13}C NMR δ (acetonitrile): 11.4, 14.5, 21.4, 23.1, 27.0, 35.6, 36.8, 43.9, 47.3, 56.9, 57.6, 62.8, 68.3, 70.3, 72.2, 74.6, 75.6, 75.7, 77.0, 79.0, 81.3, 81.8, 84.4, 92.8, 99.9, 128.2, 128.3, 128.7, 129.4, 129.5, 129.6, 130.9, 132.5, 134.4, 135.2, 137.0, 139.0, 139.9, 166.8, 168.1, 171.5, 173.6, 212.0.

Reduction of Dialdehyde to the Diol

A total of 100 mg of dialdehyde was dissolved in a 1 : 1 mixture of methanol and acetic acid and 100 mg of NaCNBH_3 was added and this mixture was stirred at room temperature for 1 hour. At this point the mixture was diluted with ethyl acetate and water and partitioned. The organic layer was washed twice with saturated NaHCO_3 solution and twice with water. The organic layer was then dried with Na_2SO_4 and evaporated under reduced pressure. The solid residue was purified by running a quick silica column with

using 40-50% acetone in benzene as the mobile phase. Evaporation of the appropriate fractions yielded 182 mg of amorphous solid as the product. UV λ_{max} (CH₃OH): 229 nm, FABMS *m/z*: 916 (M+1), 661, 653, 551, 509, 176, 154, 136, 105, 81. ¹H NMR δ : 1.07 (s, 3H, 17-H), 1.16 (s, 3H, 16-H), 1.76 (s, 3H, 19-H), 1.82 (s, 3H, 18-H), 1.82 (m, 1H, 14-H β), 1.96 (m, 1H, 6-H β), 2.24 (m, 1H, 14-H α), 2.35 (s, 3H, 4-OAc), 2.69 (m, 1H, 6-H α), 3.38-3.55 (m, 6H, 2''-, 4''-, 5''-H), 3.84 (d, 6.3Hz, 1H, 3-H), 4.13 (m, 1H, 7-H), 4.14 (d, 8.1Hz, 1H, 20-H β), 4.28 (d, 8.1Hz, 1H, 20-H α), 4.56 (br s, 1H, 1''-H), 4.80 (br s, 1H, 2'-H), 4.91 (d, 9.0Hz, 1H, 5-H), 5.23 (s, 1H, 10-H), 5.63 (d, 6.6Hz, 1H, 2-H), 5.74 (d, 6.9Hz, 1H, 3'-H), 6.17 (t, 8.4Hz, 1H, 13-H), 7.30-7.52 (m, 11H, m-OBz, m-p-NBz, o,m,p-Ph, N-H), 7.61 (t, 7.2Hz, 1H, p-OBz), 7.73 (d, 7.5Hz, 2H, o-NBz), 8.10 (d, 7.2Hz, 2H, o-OBz). ¹³C NMR δ : 10.7, 14.2, 20.7, 22.5, 26.6, 35.5, 35.7, 43.1, 46.6, 55.2, 57.0, 61.8, 62.3, 67.7, 72.1, 73.2, 74.6, 74.7, 76.5, 78.6, 78.8, 80.9, 84.0, 103.9, 127.0, 127.1, 128.2, 128.3, 128.6, 128.7, 128.9, 129.2, 130.1, 131.9, 133.7, 136.0, 138.0, 138.2, 166.8, 167.5, 170.7, 172.9, 210.3.

General Procedure for Reductive Aminations

A total of 500 mg of the dialdehyde and 500 mg of the amine were dissolved in 6 ml of 2 : 1 methanol and acetic acid and excess (200 mg) of NaCNBH₃ was added. The mixture was stirred at room temperature for 1.5 - 2.0 hours and then diluted with water and extracted three times with ethyl acetate. The organic layer was then washed with NaHCO₃ twice and with water twice. The organic layer was then dried with Na₂SO₄ and evaporated. The product was separated by putting the residue on a silica column and eluted with 20-40% acetone in benzene.

p-Aminobenzoic acid product (160)

Grayish white crystalline powder, 478 mg, mp 198-200° C, UV λ_{\max} (CH₃OH): 228, 300 nm, FABMS m/z: 1017 (M+1), 794, 754, 715, 714, 610, 206, 105. ¹H NMR δ (some DMSO): 1.11 (s, 3H, 17-H), 1.21 (s, 3H, 16-H), 1.80 (s, 3H, 19-H), 1.81 (m, 1H, 14-H β), 1.89 (s, 3H, 18-H), 2.04 (m, 1H, 6-H β), 2.38 (m, 1H, 14-H α), 2.43 (s, 3H, 4-OAc), 2.76 (m, 1H, 6-H α), 3.07 (dd, 4.5, 12.0Hz, 1H, 2''-H), 3.27 (m, 2H, 2'', 4''-H), 3.56 (br s, 1H, 4''-H), 3.69 (m, 1H, 5''-H), 3.90 (d, 6.6Hz, 1H, 3-H), 4.02 (m, 1H, 5''-H), 4.18-4.29 (m, 3H, 7-, 20 α -, 20 β -H), 4.56 (br s, 1H, 1''-H), 4.70 (br s, 1H, 2'-H), 4.93 (d, 9.3Hz, 1H, 5-H), 5.14 (s, 1H, 10-H), 5.65 (d, 6.6Hz, 1H, 2-H), 5.76 (d, 8.4Hz, 1H, 3'-H), 6.22 (t, 8.1Hz, 1H, 13-H), 6.85 (d, 8.1Hz, 2H, o-Ar), 7.29-7.61 (m, 11H, m,p-Bz, m,p-NBz, o,m,p-Ph), 7.86 (d, 7.2Hz, 2H, o-NBz), 7.93 (d, 8.4Hz, 2H, m-Ar), 8.12 (d, 7.2Hz, 2H, o-Bz), 8.13 (d, 8.4Hz, 1H, N-H). ¹³C NMR δ (some DMSO): 10.3, 13.8, 20.5, 22.2, 26.2, 35.2, 35.3, 42.7, 45.6, 46.1, 50.4, 55.2, 56.5, 61.0, 70.9, 73.5, 74.0, 74.5, 75.9, 77.6, 80.0, 80.2, 83.7, 98.3, 113.2, 120.4, 126.5, 127.0, 127.2, 127.9, 128.1, 128.1, 129.4, 129.6, 130.9, 131.1, 132.9, 133.9, 135.7, 137.9, 138.5, 153.0, 165.9, 166.7, 167.9, 169.9, 172.2, 209.6

p-Salicylic acid product (161)

Grayish brown crystalline powder, 378 mg, mp 182-184° C, UV λ_{\max} (CH₃OH): 230, 311 nm, FABMS m/z: 1034 (M + 2), 1033 (M + 1), 748, 730, 222, 204, 105. ¹H NMR δ (some DMSO): 1.11 (s, 3H, 17-H), 1.20 (s, 3H, 16-H), 1.79 (s, 3H, 19-H), 1.80 (m, 1H, 14-H β), 2.02 (m, 1H, 6-H β), 2.25 (m, 1H, 14-H α), 2.43 (s, 3H, 4-OAc), 2.74 (m, 1H, 6-H α), 3.08 (dd, 4.8, 12.6Hz, 1H, 2''-H), 3.26 (m, 3H, 2'', 4'', 4''-H), 3.69 (m,

1H, 5''-H), 3.90 (d, 7.2Hz, 1H, 3-H), 4.01 (m, 1H, 5''-H), 4.16-4.29 (m, 3H, 7-, 20 α -, 20 β -H), 4.54 (br s, 1H, 1''-H), 4.70 (d, 3.0Hz, 1H, 2'-H), 4.92 (d, 8.7Hz, 1H, 5-H), 5.14 (s, 1H, 10-H), 5.65 (d, 6.9Hz, 1H, 2-H), 5.76 (dd, 3.0, 8.7Hz, 1H, 6-Ar), 6.21 (t, 8.4Hz, 1H, 13-H), 6.29 (d, 2.1Hz, 1H, 2-Ar), 6.38 (dd, 2.1, 9.0Hz, 1H, 3'-H), 7.29-7.55 (m, 10H, m-OBz, m,p-NBz, o,m,p-Ph), 7.61 (t, 7.2Hz, 1H, p-OBz), 7.71 (d, 9.0Hz, 1H, N-H), 7.86 (d, 7.2Hz, 2H, o-NBz), 8.12 (d, 7.5Hz, 2H, o-OBz), 8.17 (d, 9.0Hz, 1H, 5-Ar).
¹³C NMR δ (some DMSO): 10.3, 13.8, 20.5, 22.1, 26.1, 35.2, 35.3, 42.7, 45.6, 45.7, 49.9, 55.1, 56.5, 60.8, 70.9, 73.5, 74.0, 74.4, 75.9, 77.2, 80.0, 80.2, 83.6, 98.1, 100.1, 103.1, 105.5, 126.5, 127.0, 127.2, 127.9, 128.0, 128.1, 129.3, 129.6, 131.1, 131.2, 132.9, 133.8, 135.6, 137.8, 138.4, 155.1, 163.0, 165.8, 166.8, 169.9, 171.9, 172.2, 209.5.

m-Salicylic acid product (162)

Reddish brown crystalline powder, 392 mg, mp 173-175° C (dec.), UV λ_{\max} (CH₃OH): 224, 343 nm, FABMS m/z: 1034 (M + 2), 1032 (M), 222, 204, 105, ¹H NMR δ : 1.10 (s, 3H, 17-H), 1.18 (s, 3H, 16-H), 1.82 (s, 3H, 19-H), 1.82 (m, 1H, 14-H β), 1.83 (s, 3H, 18-H), 2.07 (m, 1H, 6-H β), 2.31 (m, 1H, 14-H α), 2.36 (s, 3H, 4-OAc), 2.72 (m, 1H, 2''-H), 2.79 (m, 1H, 4''-H), 2.86 (m, 1H, 6-H α), 2.97 (m, 1H, 2''-H), 3.00 (m, 1H, 4''-H), 3.72 (m, 1H, 5''-H), 3.89 (d, 5.7Hz, 1H, 3-H), 3.99 (m, 1H, 5''-H), 4.20 (m, 1H, 7-H), 4.20 (d, 7.5Hz, 1H, 20-H β), 4.29 (d, 7.5Hz, 1H, 20-H α), 4.61 (br s, 1H, 1''-H), 4.79 (br s, 1H, 2'-H), 4.93 (d, 8.7Hz, 1H, 5-H), 5.28 (s, 1H, 10-H), 5.65 (d, 6.0Hz, 1H, 2-H), 5.78 (d, 9.0Hz, 1H, 3'-H), 6.18 (br s, 1H, 13-H), 6.80 (d, 8.7Hz, 1H, o-Ar), 7.08 (d, 8.4Hz, 1H, m-Ar), 7.21 (br s, 1H, p-Ar), 7.37 (m, 1H, N-H), 7.33-7.49 (m, 10H, m-Bz, m,p-NBz, o,m,p-Ph), 7.58 (t, 7.2Hz, 1H, p-Bz), 7.74 (d, 7.5Hz, 2H, o-NBz), 8.08 (d,

7.5Hz, o-Bz). ^{13}C NMR δ : 10.8, 14.2, 20.5, 22.5, 26.7, 35.6, 35.7, 43.1, 46.5, 49.9, 53.6, 55.2, 57.1, 62.7, 72.3, 73.2, 74.3, 74.6, 77.2, 78.5, 80.7, 81.0, 84.2, 99.4, 111.8, 117.2, 118.2, 126.9, 127.0, 127.1, 128.3, 128.6, 128.7, 128.9, 129.1, 130.1, 132.0, 133.5, 133.7, 135.9, 137.8, 138.5, 142.7, 156.7, 166.8, 167.6, 170.6, 172.2, 172.6, 209.9.

p-Nitroaniline product (163)

Yellow crystalline powder, 423 mg, mp 177-179° C, UV λ_{max} (CH₃OH): 230, 380 nm, FABMS m/z : 1003 (M), 987, 701, 638, 579, 207, 105. ^1H NMR δ : 1.10 (s, 3H, 17-H), 1.20 (s, 3H, 16-H), 1.77 (s, 3H, 19-H), 1.80 (s, 3H, 18-H), 1.80 (m, 1H, 14-H β), 2.02 (m, 1H, 6-H β), 2.29 (m, 1H, 14-H α), 2.38 (s, 3H, 4-OAc), 2.74 (m, 1H, 6-H α), 3.28 (m, 2H, 2''-H), 3.34 (m, 1H, 4''-H), 3.40 (m, 1H, 4''-H), 3.67 (m, 1H, 5''-H), 3.89 (d, 6.6Hz, 1H, 3-H), 4.01 (m, 1H, 5''-H), 4.18 (m, 1H, 7-H), 4.19 (d, 9.0Hz, 1H, 20-H β), 4.29 (d, 8.4Hz, 1H, 20-H α), 4.58 (br s, 1H, 1''-H), 4.78 (d, 2.7Hz, 1H, 2'-H), 4.90 (d, 8.4Hz, 1H, 5-H), 5.13 (s, 1H, 10-H), 5.65 (d, 6.9Hz, 1H, 2-H), 5.77 (dd, 2.7, 9.0Hz, 1H, 3'-H), 6.19 (t, 8.4Hz, 1H, 13-H), 6.78 (d, 9.3Hz, 2H, o-Ar), 7.21 (d, 8.7Hz, 1H, N-H), 7.33-7.51 (m, 10H, m-Bz, m,p-NBz, o,m,p-Ph), 7.60 (t, 7.5Hz, 1H, p-Bz), 7.75 (d, 7.2Hz, 2H, o-NBz), 8.10 (d, 7.5Hz, 2H, o-Bz), 8.14 (d, 9.3Hz, 2H, m-Ar). ^{13}C NMR δ : 10.7, 14.3, 20.6, 22.5, 26.7, 35.7, 35.9, 43.0, 46.3, 50.2, 55.2, 57.2, 60.7, 72.3, 73.3, 74.4, 74.6, 76.5, 78.6, 80.6, 80.8, 84.1, 98.2, 112.7, 125.9, 126.9, 127.0, 127.0, 127.1, 128.3, 128.6, 128.7, 128.9, 129.2, 130.1, 131.9, 133.6, 136.2, 137.9, 138.2, 138.8, 154.3, 166.8, 167.2, 170.6, 172.5, 210.2.

p-Aminobenzenesulfonamide product (164)

Light brown crystalline powder, 418 mg, mp 184-186° C, UV λ_{\max} (CH₃OH): 221, 273 nm, FABMS m/z: 1053 (M + 2), 1020, 767, 734, 241, 105. ¹H NMR δ (some DMSO): 1.09 (s, 3H, 17-H), 1.17 (s, 3H, 16-H), 1.75 (s, 3H, 19-H), 1.76 (m, 1H, 14-H β), 1.87 (s, 3H, 18-H), 1.98 (m, 1H, 6-H β), 2.18 (m, 1H, 14-H α), 2.40 (s, 3H, 4-Oac), 2.72 (m, 1H, 6-H α), 3.10 (m, 2H, 2''-, 4''-H), 3.25 (m, 2H, 2''-, 4''-H), 3.67 (m, 1H, 5''-H), 3.87 (br s, 1H, 3-H), 4.02 (m, 1H, 5''-H), 4.05 (m, 1H, 7-H), 4.08-4.22 (m, 2H, 20 α -, 20 β -H), 4.57 (br s, 1H, 1''-H), 4.66 (br s, 1H, 2'-H), 4.92 (br s, 1H, 5-H), 5.13 (s, 1H, 10-H), 5.61 (br s, 1H, 2-H), 5.73 (d, 7.5Hz, 1H, 3'-H), 6.17 (br s, 1H, 13-H), 6.89 (d, 8.7Hz, 2H, o-Ar), 7.28-7.61 (m, 11H, m,p-OBz, m,p-NBz, o,m,p-Ph), 7.75 (d, 8.7Hz, 2H, m-Ar), 7.87 (d, 6.9Hz, o-NBz), 8.10 (br s, 2H, o-OBz), 8.31 (d, 8.7Hz, 1H, N-H). ¹³C NMR δ (some DMSO): 9.9, 13.3, 20.1, 21.8, 25.8, 34.7, 34.8, 42.3, 45.1, 45.8, 49.9, 55.0, 56.0, 60.3, 70.2, 73.2, 73.6, 74.1, 75.4, 76.5, 79.7, 83.2, 97.7, 113.1, 126.2, 126.6, 126.7, 126.8, 127.4, 127.6, 127.7, 129.2, 130.6, 132.1, 132.4, 133.5, 135.4, 137.1, 138.3, 151.8, 165.2, 166.2, 169.4, 171.8, 209.0.

Synthesis of Glucose Pentaacetate

A total of 1.0 g of d-glucose was added to a mixture of 5.0 g (4.6 ml) of acetic anhydride and 6.5 g (6.65 ml) of pyridine at 0° C and this was stirred overnight while allowing the mixture to warm to room temperature. At this point about 20 ml of ice water was added and the product crystallized out of solution. These crystals were filtered, washed with water, and dried under reduced pressure. A total of 2.12 g of product was obtained. White crystalline powder, product existed as a mixture of α and β anomers as

determined by ^1H and ^{13}C NMR spectroscopy. Selected ^{13}C NMR signals δ : 61.4, 67.7, 67.8, 69.1, 69.7, 70.2, 72.6, 72.7, 89.0, 91.6.

Synthesis of 1 α -Bromo-Tetraacetyl Glucose

A total of 3.0 g of glucose pentaacetate was dissolved in 6 ml of CH_2Cl_2 and then 8 ml of 30% HBr in acetic acid was added and this was stirred at room temperature for 2 hours. At that point the mixture was diluted with 30 ml of CH_2Cl_2 and 75 ml of ice water and partitioned. The organic layer was separated and the aqueous layer was partitioned again with CH_2Cl_2 . The combined organic layers were washed with ice cold NaHCO_3 solution three times and then dried with Na_2SO_4 . The solvent was removed under reduced pressure and the residue was taken up in diethyl ether and ligroin and put in the freezer for crystallization. After overnight crystals had formed and they were filtered, washed with ligroin, and dried under reduced pressure. The yield was 2.20 g and this material was kept in the freezer to avoid decomposition.

Synthesis of 1-Hydroxy-Tetraacetyl Glucose

A total of 2.0 g of acetobromoglucose was dissolved in 5 ml of acetonitrile and 1 ml of water was added as excess $\text{Hg}(\text{CN})_2$ and the mixture was stirred at room temperature for 30 minutes at which time the reaction was complete. After filtering off the $\text{Hg}(\text{CN})_2$ the acetonitrile was removed under reduced pressure and the residue was diluted with water and CH_2Cl_2 and partitioned. The organic layer was separated and the water layer was partitioned again with CH_2Cl_2 . The combined organic layers were then washed with water, dried with Na_2SO_4 , and evaporated to yield 1.7 g of a syrup. Clear, colorless syrup, product existed as a mixture of α and β anomers, but the α anomer is predominate.

Selected α anomer ^{13}C NMR signals δ : 61.9, 67.0, 68.4, 69.8, 71.1, 90.0. Selected β anomer ^{13}C NMR signals δ : 60.4, 68.4, 71.9, 72.2, 73.0, 95.4.

Synthesis of 1 α -Trichloroacetimidate-Tetraacetyl Glucose

A total of 2.2 g of 1-OH-tetraacetyl glucose, 4.14 g (5 eq) of trichloroacetonitrile, and 4.4 g (1.1 eq) of K_2CO_3 was added to 4 ml of CH_2Cl_2 and stirred at room temperature for 3 days. The mixture was then diluted with water and CH_2Cl_2 and partitioned. The organic layer was separated and the water layer was partitioned again with CH_2Cl_2 . The combined organic layers were washed with water, dried with Na_2SO_4 , and evaporated to a syrup. The product was sufficiently pure for further reactions and the yield was 1.96 g. Clear colorless syrup, ^{13}C NMR δ : 20.5, 20.6, 20.6, 20.7, 61.9, 67.1, 68.5, 69.9, 71.1, 90.0, 163.8, 169.7, 170.2, 170.3, 170.9.

Synthesis of Tetraacetyl Phenyl Thioglucoside

A total of 500 mg of acetobromoglucose, 200 mg (1.5 eq) of thiophenol, and 1.25 eq of KOH were mixed in methanol with the acetobromoglucose added last. The mixture was stirred at room temperature for 20 minutes at which time TLC showed that the reaction was complete. The product crystallized upon ceasing the stirring and the crystals were filtered, washed with aqueous methanol, and dried under reduced pressure. The yield was 410 mg. White crystalline powder, mp, ^1H NMR δ : 1.99 (s, 3H, OAc), 2.02 (s, 3H, OAc), 2.08 (s, 3H, OAc), 2.09 (s, 3H, OAc), 3.73 (m, 1H, 5-H), 4.20 (m, 2H, 6-H), 4.72 (d, 6.6Hz, 1H, 1-H), 4.98 (t, 10.2Hz, 1H, 2-H), 5.05 (t, 9.9Hz, 1H, 4-H), 5.23 (t, 9.3Hz, 1H, 3-H), 7.32 (m, 3H, o,p-Ar), 7.50 (m, 2H, m-Ar). ^{13}C NMR δ : 20.5, 20.7, 62.1, 68.2, 70.0, 74.0, 75.8, 85.7, 128.4, 128.9, 131.6, 133.1, 169.2, 169.3, 170.1, 170.5.

Synthesis of Tetraacetyl Glucose, Phenyl Sulfoxide

A total of 250 mg of the phenyl thioglucoside was dissolved in 2 ml of CH_2Cl_2 at 0°C and an equivalent of mCPBA was added and the mixture was stirred at 0°C for 1 hour. The mixture was then diluted with 0.1 N NaOH and CH_2Cl_2 and partitioned. The organic layer was washed with water, dried with Na_2SO_4 , and evaporated at which time the product crystallized. After drying under reduced pressure the yield was 226 mg. Yellowish white crystalline powder. The product existed as a almost equal mixture of α and β anomers with anomeric carbon signals at 89.8 and 92.2 ppm.

Preparation of Tetraacetyl Benzyl β -Glucoside by the Koenigs-Knorr Method

A total of 300 mg of acetobromoglucose and 80 mg of benzyl alcohol was dissolved in 2 ml of CH_2Cl_2 and excess ZnCl_2 was added (100 mg). The reaction was stirred at room temperature for 18 hours, the solid was filtered off and the filtrate was diluted with water and CH_2Cl_2 and partitioned. The organic layer was washed with water, dried with Na_2SO_4 , evaporated, and the residue was ran on a silica column using 30-40% ethyl acetate in ligroin. A total of 216 mg of crystalline product was upon fraction evaporation. White crystalline powder, ^1H NMR δ : 2.00 (s, 3H, OAc), 2.01 (s, 3H, OAc), 2.02 (s, 3H, OAc), 2.11 (s, 3H, OAc), 3.68 (m, 1H, 5-H), 4.18 (dd, 2.1, 12.0Hz, 1H, 6-H), 4.28 (4.8, 12.0Hz, 1H, 6-H), 4.55 (d, 7.5Hz, 1H, 1-H), 4.63 (d, 12.3Hz, 1H, OCH_2Ar), 4.90 (d, 12.3Hz, 1H, OCH_2Ar), 5.04-5.21 (m, 3H, 2-, 3-, 4-H). ^{13}C NMR δ : 20.5, 20.6, 61.9, 68.4, 70.7, 71.2, 71.8, 72.8, 99.2, 127.7, 128.0, 128.4, 136.6, 169.2, 169.3, 170.2, 170.6.

Preparation of Tetraacetyl Benzyl β -Glucoside by the Trichloroacetimide Method

A total of 100 mg of tetraacetyl glucosyl trichloroacetimide and 28 mg of benzyl alcohol was dissolved in 2 ml of CH_2Cl_2 and 36 mg of PTSA was added. The reaction mixture was stirred at room temperature for 1 hour and the mixture was diluted with water and CH_2Cl_2 and partitioned. The organic layer was washed with water, dried with Na_2SO_4 , and evaporated. The residue was separated on a silica column using 30-40% ethyl acetate in ligroin as the solvent. A total of 56 mg of product crystallized from the evaporating fractions. White crystalline powder, ^1H and ^{13}C NMR spectra chemical shifts were identical with those reported above.

Preparation of Tetraacetyl β -Sitosterol β -Glucoside by the Koenigs-Knorr Method

A total of 300 mg of acetobromoglucose and 300 mg of β -sitosterol was dissolved in 3 ml of CH_2Cl_2 and excess ZnCl_2 was added (100 mg). The reaction was stirred at room temperature for 18 hours, the solid was filtered off and the filtrate was diluted with water and CH_2Cl_2 and partitioned. The organic layer was washed with water, dried with Na_2SO_4 , evaporated, and the residue was ran on a silica column using 30-40% ethyl acetate in ligroin. A total of 168 mg of amorphous solid was upon fraction evaporation. White amorphous solid, ^1H NMR δ (only downfield signals are listed): 3.49 (m, 1H, 3-H), 3.67 (m, 1H, 5'-H), 4.11 (d, 11.7Hz, 1H, 6'-H), 4.26 (dd, 4.5, 11.7Hz, 1H, 6'-H), 4.59 (d, 8.1Hz, 1H, 1'-H), 4.95 (t, 9.3Hz, 1H, 2-H), 5.07 (t, 9.6Hz, 1H, 4-H), 5.21 (t, 9.6Hz, 1H, 3-H), 5.36 (d, 4.2Hz, 1H, 6-H).

Preparation of Tetraacetyl β -Sitosterol β -Glucoside by the Trichloroacetimidate Method

A total of 100 mg of tetraacetyl glucosyl trichloroacetimidate and 100 mg of β -sitosterol was dissolved in 3 ml of CH_2Cl_2 and 36 mg of PTSA was added. The reaction mixture was stirred at room temperature for 1 hour and the mixture was diluted with water and CH_2Cl_2 and partitioned. The organic layer was washed with water, dried with Na_2SO_4 , and evaporated. The residue was separated on a silica column using 30-40% ethyl acetate in ligroin as the solvent. A total of 62 mg of amorphous solid product was obtained from the evaporating fractions. White amorphous powder. ^1H NMR spectrum chemical shifts were identical with those reported above.

Attempted Glucosylation of 2'-Acetyl Paclitaxel by the Koenigs-Knorr Method

A total of 200 mg of 2'-acetyl paclitaxel and 200 mg of acetobromoglucose was dissolved in 3 ml of CH_2Cl_2 and 100 mg of AgNO_3 was added. This mixture was stirred overnight at room temperature. At this time the mixture was filtered and the filtrate was diluted with water and CH_2Cl_2 and partitioned. The organic layer was washed with water, dried with Na_2SO_4 , and evaporated. The residue was separated on a silica column using 15-30% acetone in benzene as the mobile phase. A total of 132 mg of product was obtained as an amorphous solid. ^{13}C NMR δ : 10.6, 11.5, 19.8, 20.4, 25.7, 27.2, 32.6, 36.2, 44.1, 53.3, 57.6, 63.5, 68.6, 69.3, 71.1, 71.2, 71.3, 72.8, 74.5, 75.1, 82.0, 126.4, 127.0, 128.2, 128.4, 128.7, 128.8, 129.6, 129.8, 131.9, 133.1, 134.2, 136.9, 137.5, 145.9, 166.3, 167.3, 167.6, 169.4, 169.6, 169.7, 202.0.

L1210 Cytotoxicity Assay

The cells are maintained and subcultured in RPMI medium that was prepared in a sterile manner in the laboratory. The cell population was maintained between 150,000 and 600,000 cells/ml. At the time of the assay, the cell suspension was diluted to contain 150,000 cells/ml. This solution (2 ml) was placed in each well of a Becton-Dickinson deep-well plate (24 wells/plate). The test compounds were weighed and dissolved in sufficient DMSO to make 2 mg/ml. Then several dilutions of these stock solutions were made and tested. To the wells that contained the L1210 cells were added 10 μ l aliquots of the DMSO solutions so that the final concentration was known in parts per million. For paclitaxel the concentrations used were 0.1, 0.05, 0.025, 0.0125, 0.00625, and 0.003125 ppm, and for all other compounds it was 10, 2, 0.2, 0.1, and 0.05 ppm. Controls were also used in which 10 μ l of DMSO were added to the well. Each concentration was tested in quadruplicate (4 wells).

After addition of the compounds the plates were incubated for 48 hours. The plates were removed and the cells in each well were counted to determine the number of cells per ml. The contents of each was thoroughly mixed using a sterile 2 ml pipette, then, 1 ml of the cell suspension was transferred to a clean test tube and diluted with 1 ml of trypan blue stain which only dyes the dead cells. The viable cell (unstained) were then counted by shaking and placing 0.1 ml of this suspension on a Fisher hemacytometer and counting the cells found in the five gridded areas of the hemacytometer. This number was then multiplied by 4000 which gave the number of cell/ml.

The IC_{50} for each case was determined from the plot of log [concentration] versus percent inhibition (not shown). The percent inhibition for each concentration was determined by the following equation:

$$\% \text{ Inhibition} = [1 - (T_d - T_o / T_c - T_o)] * 100$$

Where T_d is the number of cells per ml of the drug treated wells, T_o is the number of cells at the start of the test, and T_c is the average number of cells per ml in the control wells. The average of four readings for each concentration was used to calculate the IC_{50} for each compound.

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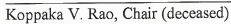
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BIOGRAPHICAL SKETCH

The author was born to James Harvey Johnson and Lawilla McLamb Johnson on September 30, 1970 in Fayetteville, North Carolina. His father worked for the civil service at Fort Bragg Army Reservation and his mother was a homemaker. He grew up with two older sisters, Melinda and Teresa. He attended and graduated in June 1988 from Cape Fear High School in Vander, North Carolina near Fayetteville. In January 1989 he enrolled at Fayetteville State University on a full academic scholarship. It was at this time that he began to develop a love for the discipline of chemistry. After graduating with a B.S. in chemistry with honors in May 1993 he enrolled in the Department of Medicinal Chemistry at the University of Florida during August 1993. It was there that this doctoral work was completed under the supervision of Koppaka V. Rao. The author was married to Amy Raye Hardy on April 30, 1994 and has one child, James Harvey Johnson III (Trey) born September 23, 1995.

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Professor of Medicinal Chemistry

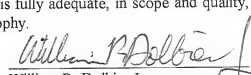
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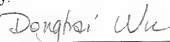
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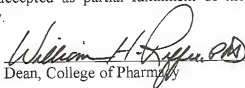
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This dissertation was submitted to the Graduate Faculty of the College of Pharmacy and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

December 1998


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